The Journal of Allergy

VOL. 28 NOVEMBER, 1957 No. 6

Original Articles

MECHANISMS OF IMMUNOLOGIC TISSUE INJURY

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MY PLAN is first to discuss the sites of antigen-antibody interaction and then discuss in more detail the possible role of the mast cell in allergic inflammation.

First of all, I should like to say a few words about how antigens and antibodies may get into cells. This has always been a great puzzle except in the case of cells that are phagocytic in the usual sense. There is, of course, no difficulty in realizing how macromolecules get into these cells. In the case of cells which are not phagocytic, however, this has been difficult to understand. With regard to this phenomenon, Dr. Stanley Bennett,[1] has recently presented a concept which I believe to be a very important one, having to do with the manner in which macromolecules are taken up by cells. The basis of this concept was originally introduced by Lewis.[2] It was recently reintroduced by Palade,[3] and has lately been elaborated on by Bennett.[1] Perhaps the best terms to describe this phenomenon are *microphagocytosis* or *pinocytosis* (''to drink''), as suggested to Lewis[2] by Professor Robinson. This property of cells has been referred to as the Lewis phenomenon. It has been observed that many different types of cells have surface membranes which are very active, that invaginations of these membranes occur, and that these invaginations frequently pinch off inside the cell to form vesicles.[4, 5] Substances may be ingested in this manner; that is, the substance becomes attached to the cell surface, possibly by specific binding groups, invagination then occurs, the invaginated portion pinches off, and the internal vesicle is formed. These vesicles apparently may be carried to the opposite side of the cell and evaginated in the same manner. Bennett believes that this very important vital process occurs in a great many cells and that the process accounts for the transfer of macromolecules and, indeed, smaller

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Based on an informal address presented at the thirteenth annual meeting of the American Academy of Allergy, Los Angeles, California, Feb. 4, 1957.

Received for publication Sept. 25, 1957.

molecules across cell boundaries. Thus, substances may pass completely through the cell without the presence, at any time, of an actual pore or break in the cell membrane.

This impresses me as being one of the most important new concepts in cytology in the last fifty years. It serves to explain an observation which has long puzzled immunologists, namely, how foreign antigens of high molecular weight get into cells.

The histochemistry of injected antigens recently has been reviewed and summarized by Coons.[6] At the time of the first work with fluorescent antibody by Coons and associates, many of us were surprised to learn that injected foreign antigens actually get into cells all over the body, even into cell nuclei, in a matter of minutes. This was a great surprise because many of these cells (for example, the hepatic cells of the liver and many others) are not generally considered to be "phagocytic" in the usual sense.

According to Bennett's concept of pinocytosis, we have a mechanism for explaining not only how large molecules get into cells, but also why their entrance may exhibit selectivity. Not only antigen and antibody, but also complexes of antigen and antibody, could readily get into cells by this mechanism.

I should like to stress that there is some evidence for elective localization of certain antigens.[6-8] Antigens of various sorts, when injected into animals, are not necessarily distributed evenly within various types of cells. Selectivity may be determined by the mechanism of phagocytosis[9-12] as well as by pinocytosis. The fluorescent antibody works of Coons and associates and of others, together with many ancillary observations, have established that some antigens become localized selectively in tissue. Selective localization may occur by virtue of special affinities of cells as well as differences in the vascularity of tissue. The localization of antigens is extremely important, as the cells and tissue sites at which antigens become fixed and persist may determine in great measure the subsequent allergic responses and the sites of lesions. Of course, as I will point out later, it is possible that antibodies as well may become selectively localized in tissues by cells which do not produce them.

I think of such diseases as rheumatic fever as resulting from selective localization of antigens; I favor the concept that in the case of rheumatic fever the streptococcus growing in the throat produces the causative substance, which I consider an antigen, since I believe this disease to be allergic in nature. I believe that this antigen gets into the circulation, is carried throughout the body, and selectively localizes in certain cells or tissue sites and, moreover, that when antibodies are formed against it they, in turn, circulate to reach and react with residual fixed antigen to produce the local lesions. Alternatively, one does not have to suppose that the causative antibody need be formed at a site distant from the lesions, for it may be formed locally. Indeed, in various lesions of rheumatic fever one may occasionally see plasma cells.[13] Plasma cells may mean different things to different persons. To me, the presence of plasma cells means that antibody formation is taking place and, moreover, that it is

taking place locally. There is no reason to doubt that an antigen at a local site can stimulate the formation of antibody at that site which, in turn, can react with it to produce the local lesion.

I was greatly interested in the report by Dr. Frank Dixon, in which he showed by the fluorescent antibody technique that gamma globulin is present in large amounts in the lesions of rheumatic fever, glomerulonephritis, and polyarteritis nodosa. It seems highly probable that the gamma globulin in such lesions is specifically bound to antigen and is truly antibody. There is urgent need of more work to explore this concept.

Now, with respect to antibody and its localization, we must remember that there are many different types of antibody. We think first, of course, of ordinary circulating antibody, comprised of complete and incomplete varieties. There are also reagin, often termed the Prausnitz-Küstner (P-K) type antibody, and the "antibody" of delayed hypersensitivity which I prefer to call the antibody of tuberculin type hypersensitivity.

We know very little about where these various types of antibody localize with respect to cells. For example, we know nothing, so far as I am aware, about the cellular localization of the P-K or reagin type of antibody. Such information would appear to be highly desirable, for it is probable that we will not have nearly all the answers to the problem of tissue injury until we know exactly where antigen and antibody are distributed, where they unite, and how such union at specific sites disturbs the functions of cells and tissues. The recent development of tracer techniques should spur investigation of this problem.

So far as gamma globulin itself is concerned, it has been shown that homologous gamma globulin is distributed widely and quite uniformly to many different cells and tissues of the body.[14] We know very little,, however, with respect to antibodies that may have properties which differ from nonantibody gamma globulin, such as high molecular weight polymers of gamma globulin or those, such as the P-K antibody, which may belong to a different molecular species.

With respect to the cellular sites at which antigen and antibody unite, one can visualize that they may unite at the surface of the cell and/or within the cell and, moreover, whether they unite at the surface or within the cell may have a great influence on the resultant irritation or injury to the cell.

It seems likely that antigen and antibody may often unite at the surface of mammalian cells without producing injury. For example, in in vitro systems antigen and antibody can unite at the surface of cells without apparent destructive effects, even in the presence of complement.[15] In other instances destructive effects are observed only in the presence of complement.[16] In the case of bacteria, it is notable that only the gram-negative bacteria are killed by antibody, and only when complement is present. Indeed, it may be that, when antigen and antibody unite in vitro at the surface of mammalian cells, it is only when complement or certain of its components are present that cell destruction is likely to occur.[17-20] There may be exceptions to this.[21, 22] Such exceptions

may also occur in vivo; for example, as I recall, Castle[23] has shown that anti-red cell antibody can produce intravascular hemolysis, presumably by mechanisms not involving complement.

It is very difficult to define the role which complement may play in a great many of the in vivo antigen-antibody reactions. Indeed, many investigators, particularly Osler and his associates,[24] are actively investigating the possible mechanisms to account for the role which complement may play in injurious reactions in the body that result from antigen-antibody interaction.

It is certain that complement levels frequently drop during allergic episodes,[25-28] but we know very little concerning the exact role that complement may play in such reactions.

On the matter of intracellular sites, if an antigen and an antibody react within a cell, and especially if the antigen is an integral part of the cell, one would expect that serious damage might be caused. For example, in case an antibody interacts with an antigen of mitochondria concerned with vital functions of the cell it is understandable that this could readily lead to injury. To date there is no good evidence on this point. Indeed, there are very few instances in which it appears that antigen, either foreign or native, and antibody unite within a cell. The one example that I would point to as a probability, and which I regard as a very exciting development, concerns the rejection of tissue homografts in which immunity is apparently dependent on an antigen located in the nucleus, probably a DNA complex.[29] Provided that the antigen of homograft immunity is located in the nucleus, the antibody would have to get into the cell in order to react with the antigen and produce damage.

The work of Prehn and his colleagues[30] on cancer immunity is along these same lines. By their chamber technique, they have shown that the casting-off process can be brought about only if the actual tumor tissue comes in direct contact with the cells of the immune host. This indicates not only that the antibody is cellular and of the tuberculin type, but also that it may be transferred to the cancer cell directly from some other cell. I am strongly inclined to believe that this is a tuberculin type of hypersensitivity involving a cell-fixed antibody and I should like to propose at this time the possibility that a lymphocyte, let us say, if it is the cell that carries tuberculin-type antibody, might literally "back up" to some other cell and transfer antibody to that cell by the mechanism of pinocytosis. This, I believe, would be in keeping with the theory of Bennett[1] for, as I recall, he and his students,[10] and others[31] as well, have expressed the view that macromolecules and even particulate matter may be transferred directly from one cell to another when the cell membranes lie adjacent to each other.

Next I should like to speak about secondary mediators of allergic reactions. This is probably more familiar ground to you. When antigen and antibody unite to produce tissue injury, whether such union is on the surface of the cell or within the cell, it has been generally regarded that in the majority of instances either the antigen or the antibody is in a fixed position. It is also generally believed that often the most profound tissue injury is not directly

due to injury to the cells in which the antigen-antibody interaction occurs, but is, instead, the result of "mediators" liberated by this interaction into the milieu, the tissue fluids and the blood, which, in turn, act upon other cells. Of these mediators, histamine is the best known.

Anaphylaxis has always been a classical experimental model and has been much used by immunologists to study the basis of allergy. It is generally regarded that histamine is the principal mediator of anaphylaxis of the guinea pig and of the dog. However, when it comes to other animals this is not so certain and, indeed, even in the case of the guinea pig and the dog there are many who have expressed doubt that histamine is the sole mediating agent. In a recent review Rose[32] has ably summarized the evidence indicating that histamine is not the only mediator agent in anaphylaxis. Although most of you are probably quite familiar with such reasons, I will enumerate a few. For example, it is found that papaverine will protect against anaphylaxis in the guinea pig, but not against histamine shock. We noticed a number of years ago, in work done on the role of antihistaminics in the prevention of anaphylaxis in the guinea pig, that several antihistaminics failed to prevent delayed death even though they prevented the acute phases of shock.[33] Many of the animals died of protracted shock. At post-mortem examination they did not show the classical picture of acute anaphylaxis. Indeed, the lungs were often not emphysematous at all. Instead there was some pulmonary edema, and petechial hemorrhages were present in the lung and in the serosa, especially along the gastrointestinal tract. Apparently these animals died of something other than the classical pulmonary effects seen in acute anaphylaxis and histamine shock. I also recall an in vitro experiment by Campbell and Nicoll,[34] in which they placed lung tissue of a sensitized guinea pig and the uterus of a rat in the same bath and added antigen to the bath. The lung tissue of the guinea pig contracted, and so did the uterus of the rat. Apparently some mediator was given off by the sensitized lung of the guinea pig which caused the contraction of the uterus of the rat. Since we know that the uterus of the rat is not sensitive to histamine, it must have been some other mediator.

One could go on with many, many such examples and, in addition, emphasize that many proponents of the histamine theory have had no intention of implying that histamine is the sole mediator of anaphylaxis, even in such species as the dog and the guinea pig.

Next I would like to turn to the possible role of the mast cell in allergic reactions.

I have long been interested in the so-called egg-white injury of the rat. In 1931, while working with anaphylaxis in the rat, the late Dr. Norris and I noticed the phenomenon of egg-white injury of the animal.[35] The nonsensitized control animals became ill upon the intravenous injection of egg white. Their snouts and forepaws swelled and they dripped fluid from their noses. A review of the literature revealed that the phenomenon had been noted in 1924 by Parker and Parker.[36] The mechanism by which egg white produces injury in the rat always remained in my mind as a very challenging question. What was going on in these rats?

To make a long story short, Dr. E. P. Benditt, the newly appointed head of pathology at the University of Washington, gave a lecture about a year ago in which he discussed his work on ovomucoid injury in the rat. It is a fascinating story.

Benditt found that egg-white injury in the rat is due to the destruction of mast cells in the feet and in the snouts of these animals by the action of ovomucoid.[37] It is of interest that Webb[38] had previously noted the disruption of mast cells in the peritoneum of the rat following exposure to egg white. May I hasten to say that mast cells are very abundant in these locations and that the large numbers destroyed by the ovomucoid resulted in the local liberation of considerable quantities of histamine and serotonin. By numerous very well-designed and executed experiments, Benditt showed that the agent liberated from the mast cells of the rat, and the one which was largely responsible for this local tissue injury, was serotonin and not histamine.[39] Indeed, the histamine effect was a very minor one. This major action of serotonin may be peculiar to the rat, and possibly other animals, as the rat is highly resistant to histamine and highly sensitive to serotonin.

TABLE I. DISTRIBUTION OF MAST CELLS IN VARIOUS ANIMAL SPECIES

SPECIES	ABUNDANT	MODERATE NUMBERS	SPARSE
Rat and mouse[?C, D]	Snout, feet, forestomach, tongue, small vessels, liver capsule	Peritoneum, skin	Spleen, thymus
Guinea pig[D]	Bone marrow	Gastrointestinal tract, lung, peritoneum	
Dog[B]	"Throttle" vein, liver, tongue, heart	Skin, lung	Spleen, kidney
Rabbit[A]	Liver capsule, blood		Skin, heart

[A]Michels, N. A.: The Mast Cells, in Downey, H.: Handbook of Hematology, New York, 1938, Paul B. Hoeber, Inc., vol. 1, pp. 232-372.

[B]Tredway, H. G., Lowry, O. H., Wahl, N., and Priebat, M. K.: Mast Cells as a Source of Tissue Histamine, J. Exper Med. **102**: 307-318, 1955.

[C]Benditt, E. P.: Studies on the Mechanism of Acute Vascular Reactions to Injury. I. The Relationship of Mast Cells and Histamine to the Production of Edema by Ovomucoid in Rats, A. M. A. Arch. Path. **60**: 104-115, 1955.

[D]Mota, L., Ferri, A. G., and Yoneda, S.: The Distribution of Mast-Cells in the Digestive Tract of Laboratory Animals: Its Bearing on the Problem of the Location of Histamine in Tissues, Quart. J. Microscop. Sc. **97**: 251-255, 1956.

Incidentally, the mast cell has many activities. There is increasing evidence that it functions as a unicellular endocrine and is engaged in the production as well as the liberation of heparin,[40] histamine,[41] and possibly serotonin[42] and hyaluronic acid.[43]

Benditt's work immediately excited my interest because of work that I had previously done on anaphylaxis in the mouse, in which I could never secure evidence to show that histamine was involved. Consequently we have begun some late work on anaphylaxis in the mouse with the intention of examining the proposition that serotonin may be responsible for anaphylaxis in this animal.

Before going into that, I should like to present some tables, first on the tissue distribution of the mast cell in various animals, then on the serotonin

present in the mast cells and in the platelets of various animals, and finally upon the substances liberated from injured mast cells.

Table I, which shows the distribution of mast cells in various species of animals, is based upon limited information.

In the rat the mast cells are very abundant in the snout and in the feet and there are moderate numbers in the peritoneum, where they are concentrated in the milk spots, and but few in the spleen. We find that mast cells are also abundant in the forestomach, in the tongue, about the small vessels, and in the liver capsule. This same distribution is probably also to be seen in the mouse.

Incidentally, in many species of animals the mast cells are most abundant around blood vessels, especially the blood vessels of small size. In the case of the guinea pig, mast cells are abundant in the bone marrow. Moderate numbers are present in the gastrointestinal tract, lungs, and peritoneum.

In the dog we find that mast cells are especially abundant about the throttle veins of the liver and in the tongue. There are moderate numbers in the lung and a few in the spleen. In the rabbit they are especially abundant in the liver capsule and in the blood, where they are known as basophils. It is of interest that, in contrast to other animals, most of the mast cells of the rabbit are found in the blood. There are very few in the skin of the rabbit.

To summarize, this table shows that in different species of animals there is great variation in the tissue distribution of mast cells.

Table II shows the distribution of mast cells in man. They are abundant in the uterus, thymus, bladder, tongue, skin, around large and small blood vessels, and in the digestive tract in the subserous and submucous layers. Moderate numbers are present in the medium-sized vessels, testes, heart, pancreas, spleen, etc. A few are present in the liver, kidneys, lung, adrenal, hypophysis, and placenta.

TABLE II. DISTRIBUTION OF MAST CELLS IN MAN

SPECIES	ABUNDANT	MODERATE NUMBERS	SPARSE
Man[A]	Uterus, thymus, bladder, tongue, skin, large and small vessels, intestine	Medium-sized vessels, testes, heart, pancreas, spleen, salivary, glands	Liver, kidney, lung, adrenal, hypophysis, placenta

[A]Downey, H.: Handbook of Hematology, New York, 1938, Paul B. Hoeber, Inc., vol. 1, pp. 232-372.

Table III shows the distribution of serotonin in mast cells and platelets.

Incidentally, I should say that serotonin is probably formed in the enterochromaffin cells along the gastrointestinal tract, as well as in the mast cells themselves.

Perhaps many of you are familiar with the tumor which arises from these cells, the so-called carcinoid. The argentaffin-staining material in carcinoid tumors is apparently serotonin. Persons who develop these tumors often suffer from an overproduction of serotonin and have a chronic serotoninemia.

The question marks in Table III indicate either that the substance may be present in small amounts or that information is lacking.

In the mast cells of man histamine is present, and probably a small amount of serotonin. In the platelets of man serotonin is apparently present, together with a small amount of histamine.

Both histamine and serotonin are found in the mast cells and the platelets of the dog.

TABLE III. DISTRIBUTION OF SEROTONIN AND HISTAMINE IN MAST CELLS AND PLATELETS OF VARIOUS ANIMAL SPECIES

ANIMAL	MAST CELL		PLATELETS	
Man	Histamine[D]	Serotonin?[G]	Histamine?[J]	Serotonin[B]
Dog	Histamine[F]	Serotonin[G]	Histamine[D]	Serotonin[D]
Rat	Histamine[C]	Serotonin[C]	Serotonin[A]?	
Mouse	Histamine?[I, M]	Serotonin?[G]	Histamine?	Serotonin?
Rabbit	Histamine?[I]	Serotonin?[K]	Histamine[E]	Serotonin[A]
Guinea pig	Histamine?[L]	Serotonin?[K]	Histamine[H]	Serotonin[H]

[A]Naess, K., and Schanch, S.: Effect of Reserpine on 5-Hydroxytryptamine (Serotonin) in Rabbit Blood, Acta pharmacol. et toxicol. **12**: 406-410, 1956.

[B]Shore, P. A., Pletscher, A., and others: Release of Blood Platelet Serotonin by Reserpine and Lack of Effect on Bleeding Time, J. Pharmacol. & Exper. Therap. **117**: 234-236, 1956.

[C]Benditt, E. P.: 5-Hydroxyptamine in Mast Cells, Proc. Soc. Exper. Biol. & Med. **90**: 303-304, 1955.

[D]Toh, C. C.: Release of 5-Hydroxytryptamine (Serotonin) and Histamine From Platelets by Tissue Extracts, J. Physiol. **133**: 402-411, 1956.

[E]Waalkes. P., and Weissbach, H.: In vivo Release of Histamine From Rabbit Blood by Reserpine, Proc. Soc. Exper. Biol. & Med. **93**: 394-396, 1956.

[F]Graham, H., Lowry, O., Wahl, N., and Priebat, M.: Mast Cells as a Source of Tissue Histamine J. Exper. Med. **102**: 307-318, 1955.

[G]Sjoerdsma A., Waalkes, T. P., and Weissbach, H.: Serotonin and Histamine in Mast Cells, Science **125**: 1202-1203, 1957.

[H]Humphrey, J. H., and Jaques, R.: The Release of Histamine and 5-Hydroxytryptamine (Serotonin) From Platelets by Antigen-Antibody Reactions (in vivo), J. Physiol. **128**: 9-27, 1955.

[I]Riley, J. F., and West, G. B.: Skin Histamine; Its Location in the Tissue Mast Cells, Arch. Dermatol. **74**: 471-478, 1956.

[J]Code, C.: Histamine in Blood, Physiol. Rev. **32**: 47-63, 1952.

[K]Parratt, J. R., and West, G. B.: Influence of Age on Tissue Histamine and 5-Hydroxytryptamine, Proc. Physiol. Soc. **133**: 71-72, 1956.

[L]Feinberg, S. M., and Sternberger, L. S.: Action of Histamine Liberator Compound 48/80 in the Guinea Pig, J. ALLERGY **26**: 170-179, 1955.

[M]West, G. B.: Histamine in Mast Cell Granules, J. Pharm. & Pharmacol. **7**: 80, 1955.

Whereas it is established that considerable amounts of both serotonin and histamine are present in the mast cells of the rat, there is little information concerning the amounts of these substances which may be present in the mast cells of the mouse, rabbit, and guinea pig.

Although it is known that both histamine and serotonin are present in the platelets of the rabbit and guinea pig, the only work on the platelets of the rat and mouse deals with the presence of serotonin in the platelets of the rat.

Table IV was compiled to show the various agents which will liberate "mediators" from the mast cell. In the case of compound 48/80 given to the rat and the guinea pig, it is to be noted that in the rat histamine, serotonin, and heparin are liberated from mast cells. In the guinea pig histamine and possibly small amounts of serotonin are liberated.

It is probable that the antigen-antibody reaction in the rat and mouse results in the liberation of considerable amounts of serotonin as well as histamine. Whereas in the rabbit, dog, and guinea pig relatively large amounts of histamine may be liberated, only trace amounts of serotonin could possibly be liberated since only traces are present in the mast cells of these species.

Some spaces in the table are left blank because of lack of information.

It is a curious fact in the case of peptone shock, that, whereas in the rat shock is apparently associated with the liberation of histamine but not of heparin,[44, 45] in the dog both histamine and heparin are liberated.[46] This indicates that various mediators may be bound in the mast cells of various species in different ways.

Another thing to be stressed about the mast cell is that it is apparently one of the most easily injured cells in the body. In the case of the skin it has been shown that mere stroking will cause injury to these cells.[47] At this point, I should also emphasize that by "injury" of mast cells we mean either the liberation of granules or the actual disintegration of the cells. Apparently, in some cases there is no disintegration of the cells, but simply a loss of granules. This is referred to as "degranulation." Since degranulation occurs without apparent disruption of the cell membrane, it may be the result of the evagination act of pinocytosis.

TABLE IV. SUBSTANCES LIBERATED FROM MAST CELLS

LIBERATING AGENT	SPECIES	SUBSTANCES
48/80	Rat	Histamine, serotonin, heparin[A, K]
	Guinea pig	Histamine, serotonin[±B]
Antigen-antibody reaction	Rat	Histamine[I], serotonin?[I]
	Mouse	Histamine? serotonin?[C]
	Rabbit	Histamine (blood cells)[D]
	Guinea pig	Histamine[E]
	Dog	Histamine, serotonin?[F]
Peptone	Rat	Histamine[G]
	Dog	Histamine, heparin, serotonin?[G]
Anaphylotoxin	Rat	Histamine[H]
	Dog	Histamine[J]

[A]Riley, J. F., and West, G. B.: Tissue Mast Cells; Studies With a Histamine Liberator of Low Toxicity (Compound 48/80), J. Path. & Bact. **69**: 269-282, 1955.

[B]Feinberg, S. M., and Sternberger, L. S.: Action of Histamine Liberator 48/80 in the Guinea Pig, J. ALLERGY **26**: 170-179, 1955.

[C]Fink, Mary A.: Anaphylaxis in the Mouse; Possible Relation of the Schultz-Dale Reaction to Serotonin Release, Proc. Soc. Exper. Biol. & Med. **92**: 673-675, 1956.

[D]Code, C. F.: Histamine in Blood, Physiol. Rev. **32**: 47-63, 1952.

[E]Mongar, J. L., and Schild, H. O.: A Comparison of the Effects of Anaphylactic Shock and of Chemical Histamine Releasers, J. Physiol. **118**: 461-478, 1952.

[F]Scroggie, E., and Jaques, L. B.: The Release of Histamine and Heparin by Antigens From the Isolated Perfused Liver of the Sensitized Dog, J. Immunol. **62**: 103-115, 1949.

[G]Mota, I., and Ferri, A. G.: Action of Peptone on the Mast Cell and Histamine Content of Dog Tissues, Acta haemat. **15**: 409-416, 1956.

[H]Riley, J. R.: The Effects of Histamine Liberators on the Mast Cells of the Rat, J. Path. & Bact. **65**: 471-479, 1955.

[I]Freund, J., and Stone, S.: Arthus Reaction in the Mouse and the Rat After Intralabial Injection of Antigens, J. Immunol. **76**: 138-145, 1954.

[J]Grossberg, A. L., and Garcia-Arocha, H.: Histamine Liberation in Vitro, and Mode of Binding of Histamine in Tissues, Science **120**: 762-763, 1954.

[K]Bhattachrya, B. K., and Lewis, G. P.: The Effects of Reserpine and Compound 48/80 on the Release of Amines From the Mast Cells of Rats, Brit. J. Pharmacol. **11**: 411-416, 1956.

In some instances it has been shown that degranulation may be associated with the liberation of histamine from the cell and not with the liberation of serotonin.[48] Likewise, serotonin may be liberated without the liberation of histamine.[49]

It is possible that these substances, histamine and serotonin in the mast cell, may be present in different combinations and possibly even in different granules

within the cell. Incidentally, the granules of the mast cell are regarded by some to be specialized giant mitochondria.[40, 50] They are distinct from ordinary mitochondria and appear to possess a double limiting membrane. Higginbotham and co-workers[51] have isolated these granules. They have shown that if it is done in sucrose solutions the granules do not liberate contained substances. The dog liver granules observed by Grossberg and colleagues[52] to liberate large amounts of free histamine upon injury to the limiting membrane were apparently mast cell mitochondria.

It seems that within these giant granules both bound and free forms of certain constituents may be present. It is possible that when the granule membrane is ruptured the free form can be immediately liberated, whereas the combined form may require other agents for liberation.

In speaking of the mast cell's marked susceptibility to various agents, it should be mentioned that if one merely injects distilled water into the peritoneum of the rat, the mast cells are readily disrupted.[53] Indeed, one can inject all sorts of agents which are innocuous to other cells and cause damage to the mast cell.

I should like to leave the impression with you that the mast cell is extremely susceptible to all sorts of physical and chemical agents and, moreover, that this great susceptibility of the mast cell appears to represent a defense mechanism, since the substances liberated (serotonin and histamine) are inflammatory agents. The mast cell may thus provide a very ready trigger mechanism for the induction of inflammation with the slightest physical or chemical trauma.

Before turning to our work on the mouse, I should like to mention some early observations of Drs. Philip McMaster and Jules Freund.

McMaster and his co-workers[54] studied anaphylaxis in the mouse a number of years ago. They noted that during shock in this animal swelling of the tongue, lips, and forepaws occurred. This has also been noted by Cameron.[55] McMaster also noted that careful examination of the ears and claw beds revealed a delicately sensitive and peculiar vascular reaction which was not a characteristic response to any known agent.[56, 57] He considered it to be pathognomonic of anaphylaxis in the mouse and called it the "ear vascular reaction" (EVR). The responses were essentially confined to small veins and arteries which displayed intermittent contraction. This contraction often occurred in a segmental fashion along the vessels. The reaction was found to be strictly local in origin and independent of nervous stimuli or blood pressure changes. It seems very probable to me that this was a reaction to mediators liberated from mast cells adjacent to these vessels, as large numbers of mast cells are distributed irregularly along the vessels of the ears and paws of the mouse, often being especially abundant at the bifurcation of small vessels.

Freund,[58] in a recent publication on the Arthus reaction in mice and rats, noted that one can produce this reaction very readily if he injects the antigen into the lips.[58] Moreover, when this is done it is noted that there is destruction

of the mast cells and intense local edema of the tissues. It is of interest that in many mice during severe anaphylactic shock we have noted that a visible swelling of the lower lip can be readily observed. I believe that in Freund's work[58] we have evidence indicating that the antigen-antibody reaction in the rat destroys mast cells locally to liberate serotonin as the active mediator of inflammation, for we know from Benditt's[39] work that serotonin is the principal agent responsible for the egg-white edema of rats associated with disruption of mast cells.

Now, of course, we still do not know where the antigen and antibody are interacting or whether the antibody is actually carried by the mast cell or carried by other cells which are injured to liberate substances, in turn, which injure the mast cell. Although the train of events in the Arthus reaction in the rat is certainly not clear, I believe that it is highly probable that serotonin plays an important role.

During these last few months we have been working on the proposition that serotonin is the principal cause of anaphylaxis in the mouse. Indeed, I should mention that after we started our work, Dr. Fink[59] in Colorado published an article supporting the same proposition. Her proposition was based on results with the Schultz-Dale reaction on the mouse uterus. She showed that the characteristic contraction of the uteri of the tested mice was inhibited only by agents which inhibit serotonin, namely, reserpine and lysergic acid diethylamide.

We began our experiments on anaphylaxis in mice by trying to protect them with various antihistaminic agents. Incidentally, we now produce almost 100 per cent fatality in our strain of mice by sensitizing with Freund's adjuvants and bovine gamma globulin. We have also succeeded in producing some 90 per cent fatality in mice passively sensitized with rabbit antisera. Of the various antihistaminic agents, only a few showed any protection and those showed only slight protection. It should be emphasized at this point that some of the "antihistaminic" agents are actually not specific for histamine, since they may also protect against serotonin.[60] We have not had the opportunity to try the antihistaminic agent, mepyramine. However, we have found that reserpine will completely block anaphylaxis in the mouse. So far as we know, the only action of reserpine which would be expected to block anaphylaxis is that of depleting body stores of serotonin.' If sensitized mice are treated for a day or so with reserpine and then challenged with antigen, it is found that they completely resist shock. In the two dozen or more reserpinized mice that we have tried so far, we have not observed severe shock or a single death. Of course, animals that are treated with reserpine are affected by it. Reserpine has a sedative action and at the time we challenged the mice with antigen they still showed the sedative effects of the reserpine. They remained sleepy following the challenge dose of antigen and showed no symptoms of shock. Despite the preliminary nature of our observations at this time, we feel that the results provide good suggestive evidence that serotonin is the principal mediator of anaphylaxis of the mouse. It is to be expected that this may likewise be true of the rat.

Our failure to reproduce the symptoms of anaphylaxis in the mouse by intravenous injection of serotonin does not detract from this hypothesis in view of the high local concentrations of mediators which would be expected in perivascular tissues following injury to mast cells.

The investigator who has recently written most about the mast cell is Riley.[61] He has found a correlation between the numbers of mast cells in tissues and histamine content in several species of animals. In fact, evidence is rapidly accumulating which indicates that the mast cell carries most, if not all, of the histamine in several species of animals. It may also carry a heavy load of other mediators in certain animal species.

I should now like to speculate a bit about what this may mean in terms of unexplained phenomena in allergy. Perhaps the local disruption of mast cells could explain the local exhaustion of tissue reactivity following an antigen-antibody reaction. In view of the variation in the distribution of mast cells among individuals of a species,[46, 61] I also wonder whether the allergic susceptibility of an individual and the shock organs and tissues involved might be influenced by the number of mast cells present in the tissues and possibly by the load of mediators which they contain. It has been shown that mast cells from different organs and different sites in the body may contain different loads of these substances.[48] Could the local abundance of mast cells rich in mediators in some instances account for the localized distribution of allergic lesions and of regional sensitivity?

The partial depletion of mediators by previous stimulation may likewise lead to a lowered reactivity of tissues. If one employs a smooth muscle which is sensitive to several antigens, it is observed that maximal responses to the successive addition of the several antigens may be obtained only if the response to the earlier antigens is not too vigorous.[62] I have wondered whether this might be explained by the possibility that, although in submaximal responses mast cells are being injured, they are not totally destroyed and depleted of mediators at any one time. Such partial liberation of mediators from mast cells can be achieved in the case of compound 48/80 treatment.[63]

There are certainly many unexplained phenomena in hypersensitivity reactions in which the mast cell may provide an answer.

The closing thought that I would like to leave with you is that "The Riddle of the Mast Cells," about which Riley[64] has written, promises to turn out eventually to be a fascinating "mast cell story." I personally regard the role of the mast cell in tissue injury as one of the most intriguing problems in allergy today.

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SEVERE ALLERGIC REACTION CAUSED BY SILK AS A CONTAMINANT IN TYPHOID-PARATYPHOID VACCINE

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BIZARRE and unexplained reactions, such as purpura, urticaria, vascular collapse, and fatal shock, which follow the parenteral administration of biologic products are not uncommon and have been reported from time to time in the literature.[1-7] Alexander,[8] in a review of the subject, states that such reactions to vaccines are "more frequently due to components of the culture media than to the infectious agent itself." Systemic reactions following the administration of typhoid vaccine which were characterized by severe asthma have not been reported in the literature. When other types of systemic reactions have occurred, they have generally followed the intravenous and not the subcutaneous administration of the vaccine.

An instance of a severe asthmatic reaction following the subcutaneous administration of typhoid-paratyphoid vaccine recently came to our attention. The patient (R. B.) was a 13-year-old boy with a past history of bronchial asthma and ragweed hay fever who had been under the care of two of the authors (R. F. and M. W.). He had received the usual immunizing injections against pertussis, tetanus, and diphtheria. In 1952 he had received a series of 3 subcutaneous injections of typhoid vaccine. In 1953 and 1954, booster doses of triple typhoid vaccine were administered uneventfully. In June, 1955, another booster dose of 0.5 ml. of typhoid vaccine was given subcutaneously. Within 10 minutes the patient, who had been free of asthma since March, 1947, began to cough, became dyspneic, and developed a severe attack of bronchial asthma. Epinephrine was administered, with immediate relief.

Several weeks after this incident a sample of the patient's blood was drawn for passive transfer studies. With the patient's serum, cutaneous sites were prepared on normal individuals. For testing purposes, 3 preparations of typhoid vaccine were obtained from pharmaceutical companies, while a fourth one was supplied by a health department laboratory. These various vaccines will be referred to as Brands A, B, C, and D.

One week after preparation, the passive transfer sites were tested with various dilutions of the triple typhoid vaccine (Brand A) which had precipitated the reaction in Patient R. B. Definite positive reactions were obtained with this product. With triple typhoid vaccine from the 3 other sources, no reactions occurred on the passive transfer sites (Table I).

Received for publication June 10, 1957.

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TABLE I. PASSIVE TRANSFER STUDIES ON SERUM R. B. WITH VARIOUS BRANDS OF TYPHOID VACCINE

RECIPIENT	TEST MATERIAL	DILUTION	RESULTS OF TESTS	
			SENSITIZED SITE	CONTROL SITE
3	Typhoid vacc. A	1:10	++	0
1, 2	Typhoid vacc. B	1:10	0	0
1	Typhoid vacc. B	None	0	0
4	Typhoid vacc. C	1:10	0	0
5	Typhoid vacc. D	1:10	0	0

0 = negative; + = slight positive; ++ = moderate positive; +++ = marked positive; ++++ = very marked positive.

Attempts were then made to identify the offending allergenic factor in vaccine A. Passive transfer tests were carried out with the materials contained in the media in which the organisms had been grown. None of these materials elicited a positive reaction. (Table II.)

TABLE II. PASSIVE TRANSFER STUDIES ON SERUM R. B. WITH CONSTITUENTS OF CULTURE MEDIA USED IN PREPARATION OF TYPHOID VACCINE BRAND A

RECIPIENT	TEST MATERIAL	DILUTION	RESULTS OF TESTS	
			SENSITIZED SITE	CONTROL SITE
3	Beef heart infusion	1:10	0	0
5	Beef	0.05 mg. N/ml.	0	0
3	Tryptic peptone	1:100	0	0
3	Peptic peptone	1:100	0	0

It was now apparent that commercial vaccine A contained an unidentified allergen and that this allergen was not present in the constituents of the media. This allergen was absent from Brands B, C, and D and was capable of eliciting passive transfer reactions on sites prepared with the serum of our patient. At this point, we learned from Company A that filtration through silk, an allergen to which our patient was skin-sensitive, was part of the procedure employed in the preparation of Brand A vaccine. We were also informed by Company A that another patient who had been injected with tetanus toxoid prepared by them had had an allergic reaction which, it was suspected, was due to silk sensitivity.[9] A positive passive transfer reaction to a test with silkworm extract (0.01 mg. N per milliliter) was then demonstrated at a site sensitized with the serum of Patient R. B. Strong positive reactions were also obtained on tests with a diphtheria toxoid preparation made by Company A. In addition, 1:10 dilutions of extracts of "new" and "old" silk filters used in the

TABLE III. PASSIVE TRANSFER STUDIES ON SERUM R. B. WITH DIPHTHERIA TOXOID AND MISCELLANEOUS MATERIALS USED IN THE PREPARATION OF TYPHOID VACCINE BRAND A

RECIPIENT	TEST MATERIAL	DILUTION	RESULTS OF TESTS	
			SENSITIZED SITE	CONTROL SITE
1	Diphtheria toxoid Brand A	1:100	+±	0
5	Extract of used silk filter	1:10	++	0
1	Silkworm extract	0.01 mg. N/ml.	++±	0
6	Extract of new silk filter	1:10	+++	0
2	"Extract" of pink-red rubber plugs	1:10	0	0
3	"Extract" of gray rubber plugs	1:10	0	0

preparation of Brand A typhoid vaccine also gave positive transfer reactions. "Extracts" of the rubber plugs routinely used as a closure for the vials of Brand A typhoid vaccine were negative. (Table III.)

Cross-neutralization studies were then undertaken. (Table IV.) Sites which had been previously tested with silk-filtered typhoid vaccine A failed to react when retested with the same material. Sites which had failed to react to Brands B and C, which contained no silk, reacted strongly when retested

TABLE IV. CROSS-NEUTRALIZATION STUDIES ON SERUM R. B. WITH SILK ANTIGEN AND TYPHOID VACCINES A, B, C, AND D

RECIPIENT	SITE NO.	INITIAL TEST		RETEST		NEUTRALIZATION BY INITIAL TEST
		ANTIGEN	RESULT	ANTIGEN	RESULT	
1,2,3	1	Vacc. A	+++	Vacc. A	0	Complete
1,2	2	Vacc. A	+++	Vacc. B	0	Complete
1,2	3	Vacc. A	+++	Vacc. C	0	Complete
1,2,3	4	Vacc. A	+++	Vacc. D	0	Complete
1/2/3	5	Vacc. A	+++	Silkworm*	0	Complete
1,2	6	Vacc. B	0	Vacc. A	+++	None
1,2	7	Vacc. B	0	Vacc. B	0	
1,2	8	Vacc. B	0	Vacc. C	0	
1,2,3	9	Vacc. B	0	Vacc. D	+++	None
1,2,3	10	Vacc. B	0	Silkworm	+++	None
1,2,3	11	Vacc. C	0	Vacc. A	+++	None
1,2	12	Vacc. C	0	Vacc. B	0	
1,2	13	Vacc. C	0	Vacc. C	0	
1,2	14	Vacc. C	0	Vacc. D	+++	None
1,2	15	Vacc. C	0	Silkworm	++	None
1,2,3	16	Vacc. D	+++	Vacc. A	0	Complete
1,2,3	17	Vacc. D	+++	Vacc. B	0	Complete
1,2,3	18	Vacc. D	+++	Vacc. C	0	Complete
1,2,3	19	Vacc. D	+++	Vacc. D	0	Complete
1,2,3	20	Vacc. D	+++	Silkworm	0	Complete
1,2,3	21	Silkworm	++	Vacc. A	+	Incomplete
1,2,3	22	Silkworm	++±	Vacc. B	0	
1,2,3	23	Silkworm	+++	Vacc. C	0	
1,2,3	24	Silkworm	+++	Vacc. D	+	Incomplete
1,2,3	25	Silkworm	++±	Silkworm	0 ±	Almost complete

*Silkworm antigen solution contained 0.01 mg. N per milliliter. Sites prepared with 0.1 ml. of serum R. B. and tested after 7 days. Intracutaneous tests made with 0.02 ml. of antigen extract. Silkworm extract prepared by Allergy Laboratory of Jewish Hospital, Brooklyn, N. Y.

with Brand A. In the same manner, when sites tested with B and C were retested with silk, positive reactions were elicited. When Brand D, which had originally given negative reactions (Table I), was used in the cross-neutralization studies, we were surprised to find that it now gave a strongly positive reaction and neutralized all of the silk reagins in the serum. The vial used in the neutralization study was not the same one used for the tests recorded in Table I. From correspondence with the manufacturer of Brand D, we learned that silk is occasionally used in their filtering procedures. The positive reaction to Brand D is in accord with the findings of Coleman[10] who encountered a case similar to our own and who, on the basis of our experience, was able to demonstrate that silk was the offender in his patient.

The neutralization studies demonstrated that the silk reagins in serum R. B. had been exhausted by the reactions to Brands A and D typhoid vaccine, but had not been affected by Brands B and C. It was apparent from these studies that substantial amounts of silk antigen are present in vaccines when silk is used for the filtering process.

Neutralization studies could not be continued to determine why the initial tests with silkworm (Table IV) failed to neutralize the sensitized sites to subsequent tests with vaccines A and D. This might have been based on quantitative factors with vaccines A and D containing more antigen than the silk extract (0.01 mg. N per milliliter). It is to be noted that the 0.01 dilution of silkworm extract did not on the initial test completely neutralize a site to this antigen (Table IV, site 25). There is also the possibility that a qualitative factor was involved and that the silkworm antigens in the extract and in the vaccines A and D were not completely identical. Since the antigenic materials needed to carry on such studies were not readily available and the outcome of these studies had no direct bearing on the general conclusions which were to be drawn, no effort was made to pursue them further.

Silkworm allergen was undoubtedly the agent which caused the constitutional reaction suffered by our patient following the injection of typhoid vaccine. We have learned that although nylon is used routinely in the filtering process by many pharmaceutical houses, a situation occasionally arises when it becomes necessary to substitute silk for nylon. The presence of this hidden antigen in some biologicals may have serious consequences since silk sensitivity is common among allergic patients. If the use of silk as a filter cannot be avoided, the presence of silk antigen in a biological product would seem to warrant a warning by the manufacturer on each package advising caution in the administration of the product to silk-sensitive individuals. The unsuspected presence of this antigen should also be made known to immunologists and clinicians who may use these materials experimentally and may obtain unexplained immunologic responses as a result of the presence of the hidden silk antigen.

SUMMARY

Reactions to silk extract and to certain biologicals which had been filtered through silk were demonstrated in the serum of a patient who had experienced a severe asthmatic attack shortly after receiving a subcutaneous injection of triple typhoid vaccine. Cross-neutralization tests showed that silk-filtered vaccines exhausted all the silk antibodies in the patient's serum, whereas those vaccines not silk-filtered did not affect the reactivity of the serum.

The silk antigen in biological products is a potential hazard to any silk-sensitive individual. The presence of this antigen in any parenterally administered biological should be properly indicated and a warning of its potential danger to silk-sensitive individuals should be included in the instructions for administration.

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SILK ANTIGEN AS A CONTAMINANT IN BIOLOGICAL AGENTS

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SEVERE immediate allergic reactions characterized by asthma, cyanosis, and shock have recently been reported to have occurred in silk-sensitive persons who were injected with biological agents which were contaminated with silk antigen. In Rosenblum's[1] case the allergic reaction occurred after an injection of triple antigen; in the case treated by Friedman and associates[2] the allergic reaction occurred after an injection of typhoid-paratyphoid vaccine; and in the three cases reported by Brown and Coleman[3] it came after injections of triple antigen, tetanus toxoid, human gamma globulin, and typhoid-paratyphoid vaccine.

The presence of silk in the biological agents resulted from the filtration of these agents through silk filters during the manufacturing process. All of the immunizing agents which had produced the allergic reactions cited above were manufactured by the same pharmaceutical company, designated Company A. The purpose of this study was to determine whether or not silk antigen was present as a contaminant in other products of this and other reliable pharmaceutical companies.

METHOD

Immunologic methods were used to determine the presence of silk antigen in various biological agents. Blood sera were obtained from two patients (D. W. and N. K.) who had experienced allergic shock reactions from biologicals containing silk antigen. These sera contained reagins to silk.[3] Sites on normal subjects were sensitized by intracutaneous injections of 0.05 ml. of these sera. Four days later separate sites were tested by the intracutaneous injection of about 0.02 ml. of various biological agents, antibiotics, and drugs. These products were manufactured by prominent pharmaceutical companies and most of them have been approved by the Council on Drugs of the American Medical Association. Most of the biological products were diluted tenfold with buffered saline prior to skin testing in order to lessen their irritating properties.

The following products from thirteen different pharmaceutical companies were tested:

Company A: Diphtheria toxoid;* tetanus toxoid;* tetanus toxoid, fluid; pertussis vaccine; diphtheria and tetanus toxoids;* diphtheria and tetanus toxoids* and pertussis vaccine (triple antigen); diphtheria toxin (Schick test); diphtheria toxin control (Schick test control);

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Received for publication Sept. 5, 1957.

*Aluminum-phosphate-absorbed or alum-precipitated.

tetanus antitoxin; typhoid-paratyphoid vaccine; catarrhalis combined vaccine; typhus vaccine; cholera vaccine; influenza virus vaccine; mumps vaccine; crude liver; heparin; thiamine chloride; human gamma globulin; B_{12}; potassium penicillin G; procaine penicillin G; dihydrostreptomycin; 1 per cent procaine hydrochloride; aminophylline.

Company B: Tetanus toxoid, fluid; diphtheria and tetanus toxoids;* triple antigen;* typhoid-paratyphoid vaccine; catarrhalis combined vaccine; polio vaccine; crude liver; human gamma globulin.

Company C: Tetanus toxoid, fluid; diphtheria toxoid;* pertussis vaccine; triple antigen;* diphtheria toxin and control (Schick test); tetanus antitoxin; typhoid-paratyphoid vaccine; polio vaccine; B_{12}.

Company D: Diphtheria toxoid;* tetanus toxoid;* pertussis vaccine; diphtheria and tetanus toxoids;* triple antigen, fluid; typhoid vaccine; catarrhalis combined vaccine; polio vaccine; protamine zinc insulin; regular insulin; B_{12}.

Company E: Tetanus toxoid;* triple antigen.*

Company F: Polio vaccine.

Company G: Polio vaccine.

Company H: Heparin.

Company I: Thiamine chloride.

Company J: B_{12}; potassium penicillin G.

Company K: Mercuhydrin.

Company L: ACTH.

Company M: Decholin.

Allergenic extracts prepared by the Allergy Laboratory of The Jewish Hospital of Brooklyn were used for control tests. As controls for crude liver (Companies A and B), the following allergens were tested: hog liver, beef liver, chicken liver, pork, beef, and lamb. As controls for typhus, cholera, influenza virus, and mumps vaccines, which are made from virus grown on embryonated eggs, tests were done with egg extract containing 0.01 mg. N. per milliliter. Each subject was also tested with silk antigen and buffered saline.

As further controls to demonstrate the specificity of the reactions obtained with sera D. W. and N. K., passive transfer tests were done with tetanus toxoid* (Company A), triple antigen* (Company A), pertussis vaccine (Company A), typhoid-paratyphoid (Company A), and human gamma globulin (Company A) on sera obtained from two allergic patients which contained reagins to various pollens and foods but not to silk.

RESULTS

As shown in Table I, each toxoid, antitoxin, toxin, and triple antigen of Company A gave positive passive transfer reactions which varied from slight (+) to moderate or moderate-marked (+++). The strongest reactions were

*Aluminum-phosphate-absorbed or alum-precipitated.

obtained with tetanus toxoid. Similar biological agents from Companies B, C, D, and E gave negative reactions except for diphtheria and tetanus toxoids* (Company B) and triple antigens* (Companies B and E), which gave slightly positive passive transfer reactions. Each serum yielded strongly positive passive transfer reactions to silk antigen and negative reactions to buffered saline and to horse serum, which served as a control on tetanus antitoxin.

TABLE I. RESULTS OF PASSIVE TRANSFER TESTS WITH VARIOUS TOXOIDS, TOXINS, AND ANTITOXINS ON SITES PREPARED WITH SERA CONTAINING REAGINS TO SILK

BIOLOGICAL AGENT TESTED	PHARMACEUTICAL COMPANY	RESULTS ON SITES PREPARED WITH SERUM D.W. SUBJECT 1 TEST	SUBJECT 1 CONTROL	SUBJECT 2 TEST	SUBJECT 2 CONTROL	SUBJECT 3 TEST	SUBJECT 3 CONTROL	SERUM N.K. SUBJECT 4 TEST	SUBJECT 4 CONTROL
Diphtheria toxoid* 1:10	A	++	o	+	o				
	C					o	o		
	D					o	o		
Tetanus toxoid* 1:10	A			+++	o	++	±	++	o
	D	o	o			o	o		
	E			±	±				
Tetanus toxoid fluid 1:10	A			++	o			+++	o
	B					o	o		
	C					±	±		
Diphtheria and tetanus toxoids* 1:10	A			++	o				
	B	±	o						
	D					o	o	o	o
Triple antigen* 1:10	A			++	o	++	±		
	B	+	±			+	o		
	C	±	±			±	±		
	E	+	o			±	o		
Triple antigen fluid 1:10	D	±	±						
Diphtheria toxin 1:10 Shick test	A	++	o						
	C	o	o						
Diphtheria toxin control 1:10	A	++	o						
	C	o	o						
Tetanus antitoxin 1:10	A			+	o				
	C	o	o						
Controls									
Horse serum 1:10		o	o	o	o				
Silk 0.01 mg. N/ml.		+++	o	++	o	++	o	+++	o
Buffered saline		o	o	o	o	o	o	o	o

*Aluminum-phosphate-absorbed or alum-precipitated.

Each of eight vaccines of Company A gave positive passive transfer reactions, as shown in Table II. These included viral as well as bacterial vaccines. Similar biologicals of Companies B, C, and D gave negative reactions, except for typhoid-paratyphoid (Company B) which gave the strongest passive transfer reaction of the group.

*Aluminum-phosphate-absorbed or alum-precipitated.

TABLE II. RESULTS OF PASSIVE TRANSFER TESTS TO VARIOUS VACCINES (SERUM D.W.)

VACCINE TESTED	PHARMACEUTICAL COMPANY	SUBJECT 3		SUBJECT 4		SUBJECT 5	
		TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Pertussis 1:10	A			++	o	++	o
	C	±	±				
	D					o	o
Typhoid 1:10	D	o	o			o	o
Typhoid-paratyphoid 1:10	A			+	o	++	o
	B	+++	o				
	C	o	o				
Catarrhalis combined 1:10	A	+	o	+	o	+	o
	B	o	o				
	D					o	o
Typhus (undiluted)	A			+++	±		
Typhus 1:10	A	+	o				
Influenza virus (undiluted)	A			++	±		
Cholera (undiluted)	A			++	±		
Mumps 1:10	A			+	o		
Polio (undiluted)	B	o	o	o	o	±	o
	C	+	o	+	o	±	o
	D			o	o	o	o
	F			+	o		
	G			±	o		
Controls							
Egg 0.01 mg. N/ml.		o	o	o	o		
Silk 0.01 mg. N/ml.		++	o	+++	o	++	o

TABLE III. RESULTS OF PASSIVE TRANSFER TESTS WITH VARIOUS BIOLOGICAL AGENTS (SERUM D.W.)

SUBSTANCE TESTED	PHARMACEUTICAL COMPANY	SUBJECT 1		SUBJECT 2		SUBJECT 3	
		TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Crude liver 1:10	A	+++	o	++	±		
	B	o	o				
B_{12}	A	+	o	+	o	+	o
	C					o	o
	D			o	o		
	J					o	o
Gamma globulin, human 1:10	A	+	o	+	±	++	±
1:100	A					+	o
1:10	B	o	o			±	±
Heparin	A			o	o	o	o
	H			o	o	o	o
Thiamin chloride	A			±	±		
	I			o	o		
Insulin, protamine zinc 1:10	D			o	o		
Insulin, regular 1:10	D			o	o		
Controls							
Hog liver	o	o					
Beef liver	o	o					
Chicken liver	o	o					
Pork	o	o					
Beef	o	o					
Lamb	o	o					

TABLE IV. RESULTS OF PASSIVE TRANSFER TESTS WITH MISCELLANEOUS PHARMACEUTICAL PREPARATIONS (SERUM D.W.)

SUBSTANCE TESTED	PHARMACEUTICAL COMPANY	SUBJECT 1		SUBJECT 2		SUBJECT 3	
		TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Mercuhydrin	K	±	±				
ACTH	L			±	±		
Potassium penicillin G 50,000 units/ml.	A	o	o	o	o		
	J			o	o	o	o
Procaine penicillin G 30,000 units/ml.	A	±	±			±	±
Dihydrostreptomycin 200 mg./ml.	A			±	±		
Procaine HCl, 1%	A			o	o		
Aminophylline	A			o	o		
Decholin	M					o	o

A negative passive transfer reaction was obtained with egg antigen, which served as a control on the viruses grown on egg media. Polio vaccines of Companies B, C, F, and G gave doubtful (±) or slight (+) passive transfer reactions, and that of Company D gave a negative reaction.

Of various other biological agents, antibiotics, and drugs obtained from ten different companies, positive passive transfer reactions were obtained to crude

TABLE V. RÉSUMÉ OF RESULTS OF ALL PASSIVE TRANSFER TESTS FOR SILK SENSITIVITY WITH INJECTABLES PREPARED BY VARIOUS PHARMACEUTICAL COMPANIES

SUBSTANCE TESTED	RESULTS OBTAINED WITH INJECTABLES OF PHARMACEUTICAL COMPANY							
	A	B	C	D	E	F	G	J
Diphtheria toxoid*	+		0	0				
Tetanus toxoid*	+			0	0			
Tetanus toxoid, fluid	+	0	0					
Diphtheria-tetanus toxoids*	+	+		0				
Triple antigen*	+	+	0		+			
Triple antigen, fluid				0				
Diphtheria toxin (Schick)	+		0					
Diphtheria toxin (control)	+		0					
Tetanus antitoxin	+		0					
Pertussis vaccine	+		0	0				
Typhoid vaccine				0				
Typhoid-paratyphoid vaccine	+	+	0					
Catarrhalis combined vaccine	+	0		0				
Typhus vaccine	+							
Cholera vaccine	+							
Influenza virus vaccine	+							
Mumps vaccine	+							
Polio vaccine		±	+	0		+	±	
Liver, crude	+	0						
Gamma globulin, human	+	0						
B_{12}	+		0	0				0

Negative passive transfer reactions were obtained to heparin (A, H), thiamine chloride (A, I), regular insulin (D), protamine zinc insulin (D), Mercuhydrin (K), ACTH (L), potassium penicillin G (A, J), procaine penicillin G (A), dihydrostreptomycin (A), procaine HCl (A), aminophylline (A), and Decholin (M).

*Aluminum-phosphate-absorbed or alum-precipitated.

liver (Company A), B_{12} (Company A), and human gamma globulin (Company A), as shown in Tables III and IV. Penicillin and the control antigens for crude liver gave negative passive transfer reactions.

With the control sera, negative passive transfer reactions were obtained with each of five biological agents of Company A. These biologicals had given positive passive transfer reactions with either serum D. W. or serum N. K.

In summary, fifteen different biological agents of Company A, which included toxoids, toxins, antitoxins, vaccines, crude liver, human gamma globulin, and B_{12}, were found to be contaminated with silk antigen. There were four biologicals of Company B and one of Company E which gave positive passive transfer reactions to silk antigen. Four of five brands of polio vaccine gave small positive passive transfer reactions to silk (Table V).

DISCUSSION

There are five cases on record of severe immediate allergic reactions occurring in patients sensitive to silk who received injections of biological agents which were contaminated with this antigen.[1-3] I have knowledge of five additional unreported cases, and it is likely that there are many more such cases whose true nature has not been recognized. Allergic reactions which were reported and attributed to ordinarily innocuous substances, such as human gamma globulin, and to physiologic preparations might have been caused by silk. This might also apply to reactions caused by B_{12} and crude liver extract.

The presence of small amounts of silk antigen was detected in four of five brands of poliomyelitis vaccine. While silk filters are not listed as standard equipment in the process of preparing the vaccine,[4] the possibility exists that some of the materials which are used have at some time been passed through silk filters. For example, some companies filter distilled, deionized water through silk screens and use this water in making the vaccine.

In 250 consecutive atopic patients whom I tested intracutaneously with silk extract, there were sixty-nine who developed positive reactions, an incidence of approximately 27 per cent. There were six marked reactions (++++), fifteen moderate to marked reactions (+++), fourteen moderate reactions (++), and thirty-four slight reactions (+).

While it is not possible to estimate the percentage of reactors who are clinically sensitive to silk, it would appear that there are sufficient numbers to make allergic reactions to silk a not infrequent occurrence. It is of interest that at least nine of the ten patients who experienced allergic reactions to silk as a contaminant were atopic. This parallels the incidence of atopy in "anaphylactic" reactors to penicillin.[5]

Sensitization to silk may develop after contact with materials made of silk or following injections of silk antigen as a contaminant of biological agents. Feinberg[6] has indicated that silk sensitivity may develop as a result of cross sensitization with other insects.

The risk of dangerous allergic reactions to silk could be reduced by banning the use of silk filters in the pharmaceutical industry. As substitutes, fritted

glass filters, various forms of Seitz-type filters, and nylon and stainless steel screens are available.

At the present time, silk-sensitive patients should be warned of the possible danger of biological injections. If possible, they should be skin tested with each product before use. All atopic patients under investigation should be tested as routinely for silk as they are for horse serum.

Besides producing allergic reactions, silk antigen, which is present as an unknown contaminant in such diagnostic biologicals as the Schick test, may produce false positive or unreliable skin test reactions in silk-sensitive patients.

SUMMARY

Silk is present as a contaminant in many biological agents, including toxoids, toxins, antitoxins, and vaccines, and in human gamma globulin, B_{12}, and crude liver.

Contamination of these products with silk antigen probably occurs as a result of the use of silk filters in their preparation. The use of such filters should be discouraged, since allergic shock reactions have resulted from the injection of silk-contaminated materials into silk-sensitive persons.

Until these silk filters are eliminated, silk-sensitive patients and all atopic patients, especially those with a history of a previous reaction to a biological agent, should be skin tested with biological preparations before they are administered.

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A STUDY OF LEUKOCYTIC ANTIBODIES IN ALLERGIC PATIENTS

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CONSIDERABLE evidence has accumulated in the past ten years to support the hypothesis that plasma cells or lymphocytes are a major source of antibody production.[1, 2] Passive transfer experiments with leukocytes have provided valuable information in this field of investigation.

Urbach and Koenigstein,[3] in 1924, were the first to develop a method for the passive transfer of tissue antibodies. Using fluid from the cutaneous blisters of patients suffering from allergic dermatoses, they induced delayed type* local sensitivity to the offending allergen in normal human recipients. If no naturally occurring blisters were present, they were intentionally raised by the application of cantharides plasters.

Little progress was made in this field until 1942, when Landsteiner and Chase[4] published their observations on the cellular transfer of delayed type sensitivity in animals. Living leukocytes, taken from peritoneal exudates of guinea pigs sensitive to simple chemical compounds, were transferred to normal guinea pigs. The recipient animals were found to be specifically hypersensitive on subsequent dermal testing. Further animal experiments by Chase and other investigators[5-9] have shown that delayed type sensitivity to simple chemical compounds, to tuberculin, and to bacterial antigens may be transferred by means of leukocytes obtained from lymphatic tissue, peritoneal exudates, or peripheral blood. Cytologic observations in these studies have shown that there is a strong preponderance of lymphocytes in the cell suspensions used for transfer.[1]

In human beings, delayed type sensitivity to tuberculin and to bacterial antigens has been successfully transferred by means of suspensions of viable leukocytes, disrupted leukocytes, and leukocytic extracts.[10-13] Thus, it would appear that leukocytes contain either an antibody or some antibody precursor which is responsible for the transfer of delayed type sensitivity. Lawrence and Pappenheimer[14] have named this unknown substance *transfer factor.*

Immediate type sensitivity has been successfully transferred by viable cells in animals. Chase[15] was able to show that if he used guinea pigs highly sensitive to pieryl chloride as donors, and transferred large quantities of their splenic or lymph node cells to nonsensitive recipient animals, the latter would develop both a specific cutaneous sensitivity of the delayed type and circulating

From The Roosevelt Hospital, Institute of Allergy.

Received for publication July 1, 1957.

*In this article we have used the term *delayed type sensitivity* to denote the existence of a state of altered reactivity in an individual (animal or human) in which the interval between contact with the specific antigen and the appearance of the reaction is relatively long (that is, several hours, reaching a maximum in twenty-four to forty-eight hours). By *immediate type sensitivity,* we mean sensitivity in which the reaction follows within seconds or minutes after contact with the specific antigen.

anaphylactic antibodies. He was unable to reproduce these results with cell extracts or cells which had been disrupted by freezing and thawing. Wesslén[16] transferred to normal guinea pigs lymphocytes obtained from the thoracic duct of rabbits sensitized to horse serum. The recipient animals reacted with prompt and severe anaphylactic shock when injected with horse serum one to four days after transfer. Of particular interest is a recent publication by Dixon and associates,[17] in which the authors showed that peritoneal exudate cells, which are predominantly macrophages, and lymph node cells, which are predominantly lymphocytes, are equally capable of producing a secondary precipitating antibody response in x-radiated rabbits.

The only published works dealing with the leukocytic transfer of immediate type sensitivity from human donors to human recipients are those of Walzer and his co-workers. In 1950 Walzer and Glazer[18] reported on the transfer of viable white cells from allergic patients to the skin of normal human subjects. On challenging their transfer sites with the specific antigen five to seven days later, small positive reactions were found. While the present study was being prepared for publication, Walzer and his colleagues[19] published a second article in which they reported the transfer, from human donors to human recipients, of immediate type skin sensitivity to ragweed pollen and timothy grass pollen by means of leukocytic extracts.

The purpose of the present study was to investigate some of the immunologic properties of leukocytes and leukocytic extracts obtained from the peripheral blood of sensitive human patients. We were particularly interested in determining whether or not, by conventional passive transfer techniques, these leukocytic preparations could be shown to contain antibody or antibody precursors capable of transferring immediate type skin sensitivity to nonsensitive human recipients.

We also wished to know whether or not precipitins or anaphylactic shock-producing antibodies could be found in leukocytic extracts obtained from patients whose serum contained these types of induced antibodies.

We have included in this report the results of our experiments with the passive transfer of tuberculin sensitivity in human beings, as these studies with delayed type reactions provide an interesting basis for comparison of the results obtained with sensitivity of the immediate type.

METHODS AND MATERIALS

Separation of Leukocytes.—Our method of isolating leukocytes from peripheral blood is a modification of the techniques of Baer and associates[20] and we believe that it offers the following advantages over those previously described: (1) the technique is simple to perform and (2) preparations of white cells are obtained which are completely free of visible hemoglobin.

All possible precautions were taken to maintain a sterile technique during the preparation of the various cell extracts and cell suspensions used in this study.

Bovine fibrinogen (fraction I, Armour) is used to accelerate the erythrocyte sedimentation rate of the blood of sensitive donors. The fibrinogen as supplied by the manufacturer contains 40 to 50 per cent sodium citrate by weight, which is sufficient to prevent clotting without the addition of heparin or other anticoagulant. The fibrinogen and sodium citrate are dissolved in 0.857N saline to make a solution containing 40 mg. of fibrinogen per milliliter. Next, 5 ml. of the fibrinogen solution is drawn up into a 50 ml. syringe, and then 45 ml. of venous blood from the cell donor is drawn up into the same syringe. The syringe is inverted several times to ensure complete mixing. The mixture of blood and fibrinogen is next transferred to two 30 ml. test tubes (18 mm. in diameter) *which have been coated with a thin layer of paraffin.* The paraffin prevents large numbers of leukocytes from sticking to the walls of the test tube. (In our hands, paraffin was found to be more effective in this respect than silicone.) The blood is kept at a constant temperature of 37° C. in a water bath while the sedimentation of erythrocytes takes place. Sedimentation begins in about five minutes and is complete in thirty minutes. As sedimentation proceeds, the suspension of leukocytes in plasma, which is formed above the erythrocytes, is gently pipetted off at three-minute intervals and transferred to a wide 40 ml. centrifuge tube. The plasma suspension obtained by this method contains a large number of erythrocytes as well as leukocytes.

The suspension of cells in plasma is centrifuged at 2,000 r.p.m. for five minutes and the supernatant plasma is poured off. The cells are then resuspended in 0.857N saline and centrifuged at 200 r.p.m. for two minutes. At this latter speed, the leukocytes form clumps and sink to the bottom of the tube, while the erythrocytes remain in suspension. The erythrocyte suspension is removed with a pipette and the leukocytes are resuspended in 0.857N saline. This washing and resuspension procedure is carried out a total of four times in order to remove as much plasma and hemoglobin as possible. The last wash is followed by centrifugation at 2,000 r.p.m. for five minutes. The final yield is approximately 0.2 to 0.3 ml. of packed leukocytes, completely free of hemoglobin on visual inspection.

Preparation of Cell Extracts.—The packed leukocytes are suspended in 1 ml. of distilled water. This suspension is then alternately frozen and thawed ten times. It is frozen by immersing a test tube containing the cell suspension in a bath of dry ice and acetone, and is subsequently thawed in a water bath at 37° C. An extract is prepared by allowing the disrupted leukocytes to stand in the distilled water for twenty-four hours at 5° C. This preparation is then centrifuged at 2,000 r.p.m. for five minutes to yield a milky white extract above the packed remnants of disrupted cells (Fig. 1). The supernatant extract is poured off, and the extract is restored to physiologic salinity by the addition of 0.1 ml. of 8.57N saline. Finally, the extract is passed through a 3 ml. Seitz filter. The end product is 0.5 ml. of completely colorless* fluid having the consistency of light mineral oil.

*By performing serial dilutions with a known quantity of hemoglobin in 1 ml. of saline, it was possible to demonstrate that the solution becomes completely colorless only when there is less than 1 mcg. of hemoglobin present. Our extracts thus contained negligible quantities of hemoglobin.

Preparation of Disrupted Leukocytes by Freezing and Thawing.—The packed remnants of disrupted leukocytes which remain after the extract has been poured off are suspended in 1 ml. of 0.857N saline. This preparation is thus a suspension of disrupted leukocytes in saline in a dilution of approximately 1:5.

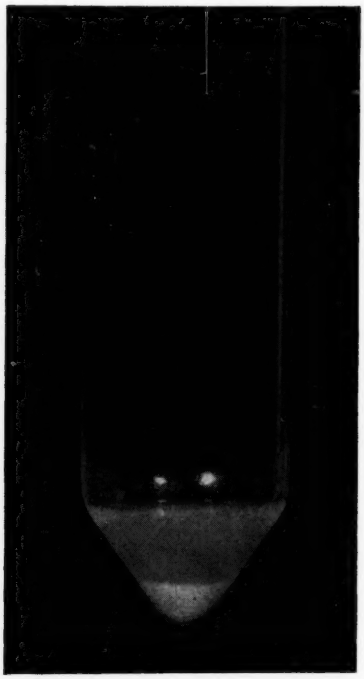

Fig. 1.—Photograph of leukocytic extract prior to filtration. Note the milky white extract lying above the packed disrupted cells.

Preparation of Disrupted Leukocytes by Mechanical Disruption.—The leukocytes are separated from peripheral blood by the method described above and are suspended in 3 ml. of 0.857N buffered saline.* Several large glass beads are added to the suspension in a test tube. The test tube and its contents are agitated over night in an Eberbach alternating shaker at the rate of 200 oscillations per minute. The leukocytes are reduced to a fine suspension of cell fragments in saline. The dilution factor in this case is 1:15.

Preparation of Suspensions of Viable Cells.—The cells are isolated in the same manner as described above, except that the initial centrifugation is done at 500 to 800 r.p.m. instead of 2,000 r.p.m., and the cells are washed three rather than four times. (Repeated washing or centrifugation at high speeds tends to reduce the number of viable cells.) The yield is about 0.2 ml. of viable leukocytes suspended in 1 ml. of buffered saline to give a dilution of 1:5.

Technique of Passive Transfer.—The recipients selected for the passive transfer experiments were either hospital personnel or professional test subjects. Two recipients were used in each passive transfer experiment. All were

*Composition of buffered saline: potassium phosphate 3.63 Gm., sodium phosphate 14.31 Gm., sodium chloride 50.00 Gm., distilled water to make 10 liters.

nonallergic persons, under 50 years of age, and in good health. Prior to the transfer they were shown to be negative on intradermal testing to the specific antigens being investigated.

The test sites were made in the skin of the arm or back by the intradermal injection of 0.1 ml. of the leukocytic material or plasma.* Plasma, cell extract, and the suspensions of disrupted cells were injected through a 26-gauge needle; a 20-gauge needle was used for the suspensions of viable cells. Little or no irritation was produced by the cell extract. The suspensions of disrupted cells and the suspensions of viable cells produced variable amounts of irritation of a minor nature at the site of injection.† In all cases, the irritation was allowed to subside before the sites were challenged with 0.05 ml. of the specific antigen. The sites injected with plasma or cell extract were challenged in forty-eight hours, those injected with cells disrupted by freezing and thawing were challenged in forty-eight to seventy-two hours, and those injected with viable cells or cells disrupted mechanically were challenged in seven days.

The passive transfer reactions designed to test for immediate type sensitivity were read in fifteen minutes. Any reaction in which, on visible inspection, there was a wheal obviously larger than the control and in which the area of erythema extended beyond the borders of the wheal was considered to constitute a positive reaction. Any lesser reaction was regarded as inconclusive evidence of a positive transfer. Positive reactions were graded from 1 plus (+) to 4 plus (++++).

The passive transfers designed to test for delayed reactions were read in forty-eight hours and graded from 1 to 4 plus. Each plus represented approximately 1 cm. in diameter.

The cell extracts and suspensions of disrupted cells were used within seventy-two hours and the suspensions of viable cells within four hours of blood withdrawal.

Test Materials.—

Tuberculin: Purified protein derivative (PPD) (Parke, Davis & Company), second test strength (0.05 mg. per milliliter), was used.

Ragweed extract: This was standardized in protein nitrogen units, according to the method of Cooke and Stull,[21] 1,000 PNU per milliliter for passive transfer testing and 100 PNU per milliliter for direct skin testing.

Penicillin: Crystalline penicillin G potassium (Pfizer), 10,000 units per milliliter, was used for passive transfer testing and 1,000 units per milliliter for direct skin testing.

Horse serum: Horse serum was diluted 1:10 for passive transfer testing and 1:100 for direct skin testing.

*The plasma used to determine levels of circulating antibody was obtained during the process of isolating the leukocytes. The plasma was defibrinogenated by centrifugation at 3,000 r.p.m. for ten minutes at 5° C.

†Our first attempts at isolating leukocytes resulted in preparations which contained significant quantities of hemoglobin. These produced considerable irritation at the transfer site. The development of a technique of preparing hemoglobin-free extracts eliminated this difficulty.

Determination of Precipitins.—The method of agar gel diffusion (Oudin) was used to demonstrate the presence of precipitins.[22] Ragweed extract (1,000 PNU per milliliter) and horse serum (diluted 1:3 in saline) were used as antigens. Normal human plasma was used as a control.

TABLE I. RESULTS OF PASSIVE TRANSFERS TO HUMAN SKIN

CELL DONOR	TEST SUBSTANCE	DIRECT SKIN TEST IN CELL DONOR WITH TEST SUBSTANCE*	CELL RECIPIENT	PASSIVE TRANSFER REACTION IN CELL RECIPIENT WITH TEST SUBSTANCE			
				PLASMA TITER	DISRUPTED LEUKOCYTES†	LEUKOCYTIC EXTRACTS	VIABLE LEUKOCYTES
S. F. N.	Ragweed	+++	J. F. R.	1:700	Neg.	Neg.	Neg.
	Tuberculin	++++	J. F. R.	Neg.	+++	+++	N. T.‡
	Ragweed	+++	A. M. A.	1:700	Neg.	Neg.	N. T.
	Tuberculin	++++	A. M. A.	Neg.	+	+	N. T.
R. R. L.	Penicillin	+++	J. B. K.	1:20	Neg.	Neg.	N. T.
	Tuberculin	++++	J. B. K.	Neg.	++	++	N. T.
	Penicillin	+++	P. G. N.	1:10	Neg.	Neg.	N. T.
	Tuberculin	++++	P. G. N.	Neg.	++	++	N. T.
J. G. E.	Tuberculin	Neg.	P. F. T.	Neg.	Neg.	Neg.	N. T.
	Ragweed	+++	J. C. Y.	1:2,000	Neg.	Neg.	N. T.
	Ragweed	+++	F. C. N.	1:1,500	Neg.	Neg.	N. T.
W. J. S.	Ragweed	++++	L. H. S.	1:750	Neg.	Neg.	N. T.
	Ragweed	++++	T. M. Y.	1:1,000	Neg.	Neg.	N. T.
G. N. T.	Ragweed	+++	M. L. E.	N. T.	Neg.	N. T.	Neg.
	Ragweed	+++	A. M. A.	1:400	N. T.	N. T.	Neg.
	Ragweed	+++	B. K. L.	1:400	Neg.	N. T.	N. T.
S. V. S.	Ragweed	+++	T. M. Y.	1:200	Neg.	Neg.	N. T.
	Ragweed	+++	R. D. S.	1:100	Neg.	Neg.	N. T.
S. N. N.	Penicillin	++++	R. D. S.	1:20	Neg.	Neg.	N. T.
	Penicillin	++++	J. B. N.	1:40	Neg.	Neg.	N. T.
A. S. O. (Day 9)§	Horse serum	++++	L. H. S.	1:80	Neg.	Neg.	N. T.
(Day 9)	Horse serum	++++	R. M. O.	1:80	Neg.	Neg.	N. T.
(Day 14)	Horse serum	++++	J. M. Y.	1:10	Neg.	Neg.	N. T.
(Day 14)	Horse serum	++++	T. M. Y.	Conc.	Neg.	Neg.	N. T.

*The reactions to ragweed, horse serum, and penicillin were of the immediate type, while those to tuberculin were of the delayed type.

†Disrupted leukocytes were prepared by freezing and thawing except for cell donor G. N. T. in which case mechanical disruption was used. A control experiment in which cells from donor S. F. N. were disrupted mechanically and injected into recipients M. L. E. and B. K. L. gave a negative ragweed passive transfer.

‡N. T. = Not tested.

§The number of days after the injection of tetanus antitoxin in horse serum sickness patient A. S. O.

Passive Cutaneous Anaphylaxis in the Guinea Pig.—Passive cutaneous anaphylaxis experiments were performed according to the technique described by Fisher and Cooke[23]; 0.1 ml. of plasma in serial dilutions or 0.1 ml. of concentrated cell extract was injected intracutaneously on the shaved belly of a guinea pig. The challenge was made six hours later with an intracardial injection of 0.3 ml. of horse serum mixed with 0.25 ml. of 1 per cent Evans blue dye per 100 grams of guinea pig body weight, or 10,000 PNU of ragweed pollen extract

mixed with 0.25 ml. of 1 per cent Evans blue dye per 100 grams of guinea pig body weight. For each case investigated, a control site of normal human plasma was placed in each test animal.

EXPERIMENTAL PROCEDURES AND RESULTS

*Tuberculin.**—Two human subjects (S. F. N. and R. R. L.), exquisitely sensitive to tuberculin on direct skin testing, were used as cell donors. Their sensitivity to tuberculin was transferred to tuberculin-negative recipients, both by cell extracts and by suspensions of disrupted cells† (Table I). The type of

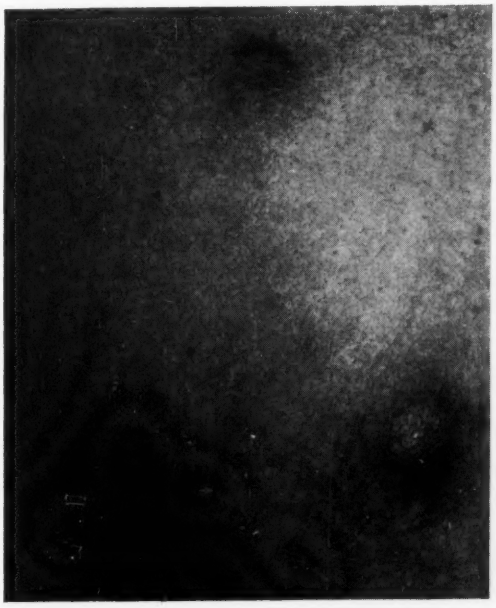

Fig. 2.—Photograph of a portion of the back of recipient J. F. R., illustrating the passive transfer of delayed type tuberculin sensitivity by means of disrupted leukocytes (upper reaction) and leukocytic extract (lower reaction). The leukocytes were obtained from tuberculin-sensitive Patient S. F. N. The photograph was taken forty-eight hours after the passive transfer sites were challenged with 0.0025 mg. of purified protein derivative.

reaction obtained at the passive transfer sites is illustrated in Fig. 2.‡ One recipient (A. M. A.) was tested subsequent to the original experiment. She was found to be tuberculin-positive one month after the passive transfer, at a site far removed from the original transfer, but was tuberculin-negative after four months. In a control experiment disrupted leukocytes and leukocytic extracts from a tuberculin-negative cell donor (J. G. E.) failed to transfer tuberculin sensitivity.

*The passive transfer experiments with tuberculin reported in this section are identical with those to be described as part of the experiments with ragweed and penicillin sensitivity (Table I). The results are recorded twice in order to avoid cross references.

†Prepared by freezing and thawing.

‡In this study leukocytic extracts and suspensions of disrupted leukocytes were injected simultaneously into the recipients. In subsequent experiments one of us (S. O. F.) has been able to transfer delayed type tuberculin sensitivity from human donors to human recipients using cell extracts alone.

Ragweed.—Five ragweed-sensitive persons with clinical symptoms of ragweed hay fever and high titers of circulating skin-sensitizing antibody were used as cell donors in these experiments (Table I).

Extracts were made from the separated peripheral blood leukocytes of four donors (S. F. N., J. G. E., W. J. S., and S. V. S.). In no case was a transfer of ragweed sensitivity obtained by means of cell extracts. Attempts to transfer ragweed sensitivity by means of suspensions of leukocytes disrupted by freezing and thawing (donors S. F. N., J. G. E., and S. V. S.), by mechanically disrupted leukocytes (donors S. F. N. and G. N. T.), or by viable leukocytes (donors S. F. N. and G. N. T.) also yielded negative results.

Patient S. F. N. is exquisitively sensitive to both ragweed and tuberculin. A leukocyte extract and a suspension of leukocytes disrupted by freezing and thawing were prepared from his peripheral blood. Four passive transfer sites were placed in the back of a tuberculin-negative, ragweed-negative recipient (J. F. R.). Two of the sites were injected with leukocytic extract and two with the suspension of disrupted cells. In forty-eight hours one cell extract site and one disrupted cell site were challenged with an intradermal injection of ragweed extract. There was no immediate reaction visible after one hour, at which time 0.05 ml. of tuberculin purified protein derivative was injected into the same sites. In forty-eight hours there were positive reactions, 2.8 cm. in diameter, at both sites (Fig. 2). No delayed reactions were found in ragweed-challenged sites not subsequently challenged with tuberculin. Thus, the leukocytic components used in this transfer were shown to be capable of transferring delayed type tuberculin sensitivity, but incapable of transferring immediate type ragweed sensitivity. This experiment was repeated, using another recipient (A. M. A.). The sites were placed in duplicate; one set was challenged with ragweed and the other with tuberculin. Those challenged with ragweed gave negative results and those challenged with tuberculin gave positive reactions, 1.5 to 2 cm. in diameter, in forty-eight hours.

A leukocytic extract from donor S. F. N. was tested for anaphylactic shock-producing antibodies and precipitins against ragweed pollen extract. It has been shown by Fisher and Cooke[23] that, in the guinea pig, an antiserum which will induce a positive passive cutaneous anaphylaxis reaction when challenged with the specific antigen will also, under the appropriate test conditions, induce a positive Schultz-Dale phenomenon and a positive passive systemic anaphylaxis reaction (anaphylactic shock). Accordingly, we have used the ability to induce passive cutaneous anaphylaxis in the guinea pig as a test for anaphylactic shock-producing antibody. The leukocytic extract was found to contain no anaphylactic shock-producing antibody to ragweed pollen extract as determined by passive cutaneous anaphylaxis, and no precipitins to ragweed pollen extract as determined by agar gel diffusion (Oudin) (Table II).

Penicillin.—Two patients (R. R. L. and S. N. N.) with clinical symptoms of urticaria, fever, and arthralgia, characteristic of an allergic reaction to penicillin, were used as cell donors. Both patients had received numerous injections of penicillin previously. Both patients gave a positive immediate direct skin

test reaction to penicillin, and their plasma transferred this reaction to human skin (Table I). Patient R. R. L. developed symptoms two days after the last injection of penicillin, and patient S. N. N. five days after the last injection of penicillin. Patient R. R. L. was also extremely sensitive to tuberculin. Attempts to transfer immediate type penicillin sensitivity by means of leukocytic extracts or suspension of leukocytes disrupted by freezing and thawing were unsuccessful, although the same leukocytic components from donor R. R. L. were capable of transferring delayed sensitivity to tuberculin (Table I).

TABLE II. COMPARISON OF THE IMMUNOLOGIC ACTIVITY OF PLASMA AND LEUKOCYTIC EXTRACT IN IMMEDIATE TYPE SENSITIVITY*

		PLASMA†			LEUKOCYTIC EXTRACT		
CELL DONOR	ANTIGEN	HUMAN SKIN-SENSITIZING ANTIBODY TITER	PCA TITER IN THE GUINEA PIG	PRECIPITINS (OUDIN)	HUMAN SKIN-SENSITIZING ANTIBODY TITER	PCA TITER IN THE GUINEA PIG	PRECIPITINS (OUDIN)
A. S. O. (Day 9)‡	Horse Serum	1:80	1:400	+	Neg.	Neg.	Neg.
A. S. O. (Day 14)	Horse Serum	1:5	1:1,600	+	Neg.	Neg.	Neg.
A. S. O. (Day 75)	Horse Serum	1:10	1:10	+	Neg.	Neg.	Neg.
S. F. N.	Ragweed	1:700	Neg.	Neg.	Neg.	Neg.	Neg.

*It should be noted that the two kinds of immediate type sensitivity are included in this table: (1) induced horse serum sensitivity (A. S. O.) and (2) spontaneous ragweed pollen sensitivity (S. F. N.).

†All experiments were done in duplicate; the PCA titer and human skin-sensitizing antibody titers are average values.

‡The number of days after the injection of tetanus antitoxin in horse serum sickness patient A. S. O.

Horse Serum.—Patient A. S. O. developed urticaria, fever, and arthralgia seven days after the injection of 1,500 units of tetanus antitoxin. A diagnosis of serum sickness (horse serum) was made. A direct skin test on the eighth day after the injection of tetanus antitoxin showed a 4 plus reaction to horse serum 1:100. Immunologic investigation of the patient's serum taken on the ninth and fourteenth days after injection of tetanus antitoxin showed high levels of skin-sensitizing antibody, anaphylactic antibody, and precipitins to horse serum (Table II). These three antibody levels were determined by means of plasma passive transfer reactions to human skin, passive cutaneous anaphylaxis in the guinea pig, and agar gel diffusion (Oudin), respectively.

It was found that leukocytic extracts, prepared from blood drawn on the same days as the serum used in the above experiments (ninth and fourteenth days) gave negative findings when tested for anti-horse serum activity by passive transfer, passive cutaneous anaphylaxis, or agar gel diffusion (Tables I and II). Disrupted leukocytes prepared by freezing and thawing at the same time the extracts were made (ninth and fourteenth days) failed to induce horse serum sensitivity in human skin (Table I).

The patient gave negative skin tests to tuberculin and other common bacterial antigens, so that it was not possible to test the leukocytic components in this case for their ability to transfer delayed type sensitivity.

Controls.—

Plasma: Plasma, obtained from ragweed-sensitive donor J. G. E. and horse-serum-sensitive donor A. S. O. during the separation of leukocytes, showed identical end points in plasma passive transfer reactions as did serum taken from the same donors at the same time.

Freezing and thawing: One milliliter of each of the above-mentioned plasmas was frozen and thawed ten times. There was no loss of skin-sensitizing antibody, anaphylactic reactivity, or precipitins, as detected by the techniques used in this study. Thus, in plasma, freezing and thawing had no disturbing effect on the antibody levels. The fact that suspensions of leukocytes prepared by mechanical disruption also gave negative results on passive transfer would seem to demonstrate that the negative results obtained with leukocytic components prepared by freezing and thawing were not due to the freezing and thawing procedure.

Distilled water: In order to show that extraction with distilled water was not responsible for the negative results, duplicate extracts and suspensions of disrupted cells were prepared from donors S. F. N. and S. V. S., using Tyrode's solution exclusively throughout the entire separation and extraction procedure. These preparations also gave negative results on passive transfer.

Wash water: The wash water which had been used for the fourth and final washing of the separated leukocytes was tested in every case for transferable or precipitable antibody, with uniformly negative findings. The second and third washes were tested in two cases (donors S. F. N. and G. N. T.). The third washes gave no positive findings, but a positive transfer to human skin was obtained with the second wash of donor G. N. T. These results, when considered together with the negative transfers of immediate type sensitivity by the leukocytic components, show that all immunologically significant traces of plasma had been removed from the preparations used and that no antibody had been washed out of the leukocytes into the wash water.

DISCUSSION

The ease with which delayed type tuberculin sensitivity could be transferred from human donor to human recipient by leukocytic extracts and suspensions of disrupted cells stands in sharp contrast to the negative results obtained in our attempts to transfer immediate type sensitivity. Our finding that the cell recipient becomes sensitive to tuberculin at a site remote from the original transfer site, and that this sensitivity persists after the transfer, is in agreement with the results reported by Lawrence. Apparently it makes little difference whether disrupted leukocytes or leukocytic extracts are used for the transfer; both preparations were found to be equally capable of transferring tuberculin sensitivity.

We do not have any explanation for these phenomena, except to note that Lawrence and Pappenheimer,[14] in their *transfer factor* hypothesis, have suggested a possible mechanism for these reactions.

The results with tuberculin transfers are also of significance in that they demonstrate that leukocytic extracts used in this study contain enough of the essential constituents of the leukocyte to duplicate one property of the intact vital cell.

Before considering the results obtained with immediate type sensitivity, it should be noted that leukocytic transfer experiments with immediate type sensitivity differ from experiments with delayed type sensitivity in one very important respect: the plasma of cell donors sensitive to tuberculin or other bacterial antigens contains no detectable antibody to those antigens, so that if a small quantity of plasma remains attached to the leukocytes it is of no significance. With immediate type sensitivity, however, it is obviously important that every trace of plasma be removed from the cells.

There is a tendency for isolated leukocytes to stick together in clumps or to attach themselves to other substances. Because of this adhesiveness, it is probable that significant amounts of plasma become attached to the leukocytes during the separation process. Therefore, dilution of the cells during the washing period to a point beyond the highest dilution of plasma which will give a positive passive transfer reaction is, in itself, no guarantee that all adherent plasma has been removed.

In our technique, the leukocytes were washed four times in 100 times their volume of saline. It has been shown that in this study no immunologically significant plasma remained attached to the cells after the final washing. However, if positive results had been obtained in the various experiments with immediate type sensitivity, it would have been much more difficult to prove that all significant plasma had been washed away from the leukocytes.

Another important factor to be considered in a study of this type is the limitation of the passive transfer method in detecting *small quantities* of human skin-sensitizing antibody. Sherman,[24] in commenting on direct skin testing in allergy, has stated: "Reading the reaction by measuring the wheal and erythema implies a precision that often cannot be proved by repetition of the tests." This remark is even more applicable to passive transfer techniques, where the number of variables is much greater than in direct skin testing.

Minor variations in the depth of the injections of sensitizing and test materials, differences in reactivity of the skin at different sites in the recipient, unavoidable leakage along the path of the needle, and lingering invisible irritation from the transferred material contribute to the lack of exactness of this method.

On the other hand, extremely minute quantities of precipitins or anaphylactic antibody may be detected with great precision by agar gel diffusion or passive cutaneous anaphylaxis, respectively.

We interpret our findings as evidence that leukocytes separated from the peripheral blood of specifically sensitive donors do not contain human skin-sensitizing antibody of the immediate type (against ragweed pollen, penicillin, or horse serum) which can be detected by conventional, carefully done passive

transfer techniques. It is possible, of course, that if some more exact method than passive transfer for detecting skin-sensitizing antibody were available, the leukocytes might be found to contain small quantities of preformed antibody. It is also possible that detectable amounts of antibody might exist in human splenic or lymph node cells, neither of which were investigated in this study.

No definite conclusion can be drawn from these results with respect to the presence of a possible human skin-sensitizing antibody precursor in the leukocytes. Because the properties of such a hypothetic substance are necessarily unknown, it can be urged that the precursor might have been destroyed in the processing of the cells.

This study has also demonstrated that leukocytic extracts from a horse-serum-sensitive donor do not contain precipitins or anaphylactic shock-producing antibody against horse serum. Unfortunately, the techniques used to detect these two types of antibody cannot be applied to the study of viable leukocytes or disrupted leukocytes.

The discrepancy between our findings and those of Walzer and his associates,[18, 19] may be due to one or more of five possible factors: (1) differences in the technique of isolating leukocytes, (2) differences in washing procedure, (3) differences in hemoglobin content of leukocytic extracts and suspensions, (4) differences in interpretation of skin tests, and (5) differences in cell donors and recipients.

SUMMARY

1. Delayed type skin sensitivity to tuberculin was transferred to tuberculin-negative human recipients by means of leukocytic extracts and disrupted leukocytes obtained from the peripheral blood of tuberculin-sensitive human donors.

2. Attempts to transfer immediate type skin sensitivity to ragweed pollen, penicillin, and horse serum from human donors to human recipients, by means of leukocytic extracts or disrupted leukocytes, were unsuccessful. Negative results were also obtained with viable leukocytes from ragweed-sensitive patients.

3. Leukocytic extracts and disrupted leukocytes obtained from patients sensitive to both tuberculin and ragweed, or to tuberculin and penicillin, are capable of transferring delayed type sensitivity to tuberculin but not immediate type sensitivity to ragweed pollen or penicillin.

4. From these findings, we have concluded that leukocytes derived from the peripheral blood of specifically sensitive human donors (a) contain an unknown substance responsible for the transfer of tuberculin sensitivity and (b) do not contain skin-sensitizing antibody against ragweed pollen, penicillin, or horse serum which can be detected on passive transfer.

5. Leukocytic extracts from patients sensitive to horse serum or to ragweed pollen did not contain precipitins or anaphylactic shock-producing antibodies against horse serum or ragweed pollen extract, respectively.

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THE EFFECT OF ω-METHOXY-POLY(ETHYLENEOXY)ETHYL p-BUTYLAMINOBENZOATE (TESSALON) ON VENTILATION AND LUNG VOLUMES

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IN BRONCHIAL asthma or pulmonary emphysema, cough, especially of the nonproductive variety, may be a vexing problem. Cough is fatiguing, it interferes with sleep, and it appears at times to add to the existing "bronchoconstriction." Unfortunately, the best of the available antitussive agents, the derivatives of morphine, have the disadvantage of depressing the respiratory center and are, therefore, best avoided in patients with possible impairment of ventilation.

TABLE I. PULMONARY AND

EXPERIMENT NO., PATIENT, AGE, AND SEX	HEIGHT (CM.)	GREATEST OBSERVED VALUES* FOR VITAL CAPACITY		BASELINE MEASUREMENTS				
		TOTAL (C.C.)	VOLUME FIRST SECOND (C.C.)	BLOOD PRESSURE	PULSE	VITAL CAPACITY TOTAL (C.C.)	VITAL CAPACITY VOLUME FIRST SECOND (C.C.)	VENTILATION (L./MIN.)
1. E. W. 28-Female	153	2,400	1,800	118/ 70	108	1,500	800	4.9
2.				108/ 78	88	2,300	1,300	14.4
3. R. G. 52-Male	178	3,550	2,600	126/ 84	64	3,300	2,500	10.2
4.				150/100	76	2,750	2,000	12.0
5. S. F. 60-Male	175	4,200	2,200	140/ 90	80	3,000	1,700	13.2
6.				124/ 88	80	3,450	1,900	12.0
7.				118/ 88	84	3,250	1,700	8.8
8. H. A. 45-Male	171	4,100	2,500	144/ 90	80	3,300	2,400	11.2
9.				132/ 84	84	3,600	2,400	6.0
10. E. H. 22-Female	165	4,600	4,000	110/ 70	84	4,400	2,300	9.0
11.				120/ 78	78	4,400	2,900	4.0
12. R. R. 29-Famale	173	3,000	1,800	104/ 80	88	2,400	1,600	11.6

*Values obtained on at least one occasion in the past 12 months.

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Received for publication Sept. 6, 1957.

In the course of animal experiments, it was observed that certain fits of coughing cease if an inspiration effort is made with the glottis closed.[1] From this, it might be inferred that an increase in stimulation of the dilation receptors occurs during inspiration and that this is important in the cough mechanism. Cough, therefore, might be avoided or lessened by any agent capable of decreasing the depth of inspiration or blocking the dilation receptors.

Fig. 1.—Tessalon.

An alkaloid-free polyglycol (Fig. 1), ω-methoxy-poly(ethyleneoxy)ethyl p-butylaminobenzoate (Tessalon) was found to be very effective in blocking the dilation receptors of the lung.[2] Preliminary clinical and pharmacologic data in subjects with various lung ailments have shown Tessalon to give effective control

CARDIOVASCULAR EFFECTS OF TESSALON

INTRAVENOUS TESSALON 10 TO 15 MINUTES AFTER ADMINISTRATION						
			VITAL CAPACITY			
DOSE (MG.)	BLOOD PRESSURE	PULSE	TOTAL (C.C.)	VOLUME FIRST SECOND (C.C.)	VENTILATION (L./MIN.)	PATIENT'S COMMENTS
2.5	116/ 70	104	1,800	1,000	6.3	"Breathing easier" Absence of "air trapping" after Tessalon
5.0	118/ 80	90	2,300	1,500	20.8	"Breathing easier"
5.0	150/ 90	72	3,100	2,500	36.0	"Breathing easier" Absence of "air trapping" after Tessalon
5.0	140/100	84	2,800	1,800	23.0	
5.0	150/100	84	3,200	1,900	32.8	Absence of "air trapping" after Tessalon
5.0	142/ 80	80	4,000	2,250	16.5	Absence of "air trapping" after Tessalon
10.0	136/ 88	84	4,250	2,550	17.0	Absence of "air trapping" after Tessalon
5.0	130/ 90	80	3,800	2,800	12.0	
5.0	130/ 90	84	4,000	2,800	10.4	"Breathing easier"
5.0	110/ 70	86	4,600	2,700	36.4	"Breathing deeper" Less resistance to breathing
7.5	120/ 80	80	4,400	3,000	4.4	
5.0	98/ 70	84	2,100	1,500	24.0	

of cough, and the drug was well tolerated.[3-5] Since cough may be only one feature of these disease states, it seemed desirable to determine the effect of intravenously administered Tessalon on the lung volumes and ventilation in patients with obstructive pulmonary disease.

TABLE II. EFFECT OF TESSALON ON COMPARTMENTAL LUNG VOLUMES

EXPERIMENT NO., PATIENT, AGE, AND SEX	HEIGHT (CM.)	GREATEST OBSERVED VALUES* FOR VITAL CAPACITY		CONTROL VALUES						AFTER INTRAVENOUS TESSALON					
								VITAL CAPACITY						VITAL CAPACITY	
		TOTAL (C.C.)	VOLUME FIRST SECOND (C.C.)	F.R.C. (C.C.)	E.R.V. (C.C.)	R.V. (C.C.)	I.C. (C.C.)	TOTAL (C.C.)	VOLUME FIRST SECOND (C.C.)	F.R.C. (C.C.)	E.R.V. (C.C.)	R.V. (C.C.)	I.C. (C.C.)	TOTAL (C.C.)	VOLUME FIRST SECOND (C.C.)
1. H. M. 32-Male	168	3,600	1,800	4,000	650	3,350	2,250	2,900	1,400	3,400	450	2,950	2,550	3,000	1,300
2. E. H. 22-Female	165	4,600	4,000	3,300	1,250	2,050	2,450	3,700	1,250	3,300	1,700	1,600	2,500	4,200	1,850
3. E. W. 28-Female	153	2,400	1,800	3,050	350	2,700	1,850	2,200	1,300	3,100	550	2,550	1,650	2,200	1,300
4. A. G. 28-Female	156	3,500	2,100	4,220	600	3,620	1,450	2,050	1,100	4,120	500	3,620	1,300	1,800	1,000
5. S. S. 32-Female	168	3,200	2,400	3,500	650	2,850	2,350	3,000	1,800	3,900	700	3,200	2,500	3,200	2,000
6. W. K. 39-Male	165	3,600	1,800	4,100	700	3,400	1,850	2,550	1,050	4,400	400	4,000	2,200	2,600	1,050

*Values obtained on at least one occasion in the past 12 months.

METHODS AND MATERIALS

Ten patients with bronchial asthma of five or more years' duration served as subjects for this study. The sexes were evenly divided, and the ages ranged from 22 to 60 years.

The subjects were seated and connected by means of a soft rubber mouthpiece to a closed spirometric system recording on two kymographs moving at different speeds, one continuously at 5 cm. per minute and the second intermittently at 60 cm. per minute. Oxygen was supplied into the system to replace that consumed by the subject and carbon dioxide was absorbed from the system by means of a soda-lime scrubber. Respirations were recorded throughout the study, and intermittently the vital capacity and expiratory flow rate were recorded on the faster-moving kymograph. In six patients the functional residual capacity (F.R.C.) and residual volume (R.V.) were determined by means of a helium dilution technique described previously.[6] The inspiratory capacity (I.C.) and expiratory reserve volume (E.R.V.) were also measured. All studies were completed prior to and within ten to fifteen minutes after the rapid intravenous injection of Tessalon in the dosage range of 2.5 to 10 mg.

RESULTS

The results are shown in Tables I and II. In nine of the eighteen completed studies the vital capacity and the volume in the first second increased by 200 c.c. or more following Tessalon. In eight there was little or no change and, in the remaining one the volume in the first second increased by 200 c.c. without a change in vital capacity.

Respiration increased in both depth and volume in most instances, with a one- or twofold increase in the minute volume (Table I).

Among the six patients in whom the F.R.C. was measured (Table II), only one showed a significant decrease (600 c.c.) in this volume following Tessalon; in three patients the decrease was less than 100 c.c.; and in two a slight increase was observed. No consistent effect was observed on the inspiratory capacity, the expiratory reserve volume, or the residual volume.

The effect of Tessalon on the cardiovascular system was variable. In four patients a rise in blood pressure was observed. This increase, for the most part, appeared to be transitory, disappearing, as a rule, within ten or fifteen minutes. No significant change in pulse rate was noted in any patient. The tolerance for intravenously administered Tessalon, in the dosage given, appeared to be excellent and no side effects were noted.

DISCUSSION

The site of action of Tessalon appears to be at the peripheral nerve endings of the lung, those which mediate the Hering-Breuer reflex.[2] If Tessalon acts in this manner to decrease active inspiration, one would expect that the inspiratory capacity would be decreased in the studies that we carried out. This

was not apparent by the technique employed. Such changes as were observed could not be attributed specifically to an alteration in any one fraction of the vital capacity or in the residual volume.

Since the minute volume increased markedly in ten of the twelve studies, it would be reasonable to assume that Tessalon, given intravenously, has a central stimulating effect on respiration. Despite the sharp increase in both the rate and depth of respiration in these patients with bronchial asthma, no embarrassment to breathing was noted. Indeed, the majority of the patients described subjective improvement in breathing. Nevertheless, there is little to indicate that intravenous Tessalon, in the dosage used, has significant bronchodilator activity.

SUMMARY

Tessalon, ω-methoxy-poly(ethyleneoxy)ethyl p-butylaminobenzoate, was given intravenously to ten patients with bronchial asthma. Ventilation and lung volumes were measured prior to and after the administration of the drug. No significant alteration in lung volumes or expiratory flow rate was observed. Tessalon, however, consistently increased the minute ventilation and, at the same time, appeared to provide patients with a sensation of ease in breathing. No objective basis for this was observed.

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OBSERVATIONS ON ACQUIRED SKIN-SENSITIZING ACTIVITY BY CLINICALLY NONALLERGIC PERSONS TO EXTRACTS OF RAGWEED POLLEN

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IN A previous article[1] it was shown that the water-soluble extract of ragweed pollen* (WSR) could be separated by electrophoresis on paper into four allergenically active fractions (designated AA_1 + Pt, P_3, P_2, and P_1 + C) and three inactive fractions. The relative activity of the fractions† tested by the passive transfer method of Prausnitz and Küstner is listed in Table I. As described in another article,[2] fraction P_3 was the most active, fraction P_1 + C was the least active, and fractions AA_1 + Pt and P_2 displayed intermediate and almost equal activity. Only persons without family histories of allergy, who gave no reactions to intradermal injections of WSR, were accepted as suitable subjects for passive transfer tests.

The passive transfer experiments to be reported here were performed in two stages. In the first stage nine sites were sensitized with undiluted allergic serum (0.05 c.c. of serum per injection). In the second stage of the experiment (24 hours later) sites 1 to 3, 4 to 6, and 7 to 9 were injected with fractions AA_1 +Pt, P_3 and P_2, respectively, at intervals of twenty-four hours until neutralization was achieved with respect to each of these fractions. This stage usually required four to five days. Each injection consisted of 0.05 c.c. of allergen solution.

One to four days after the initial injection of the allergen (ragweed) fractions, it was noted that positive reactions to WSR could be elicited on unsensitized portions of the backs and arms of some subjects. When all the fractions were tested in these persons, the largest reactions were elicited to fraction P_1 + C (Table II) and lesser reactions were produced to fraction P_2. Surprisingly, no reactions were produced to fractions AA_1 + Pt and P_3, except in one subject (M. D.) who gave a moderate reaction to P_3. In addition, all of these subjects gave reactions (2+ to 3+) to WSR. The results of these experiments are summarized in Table II.

From the above results, one would be inclined to conclude that the newly acquired reaginic activity by the subjects used for passive transfer was not due

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To be submitted in partial fulfillment for the degree of Ph.D. (M. Richter) in the Department of Biochemistry, McGill University.

Supported by a grant from the Department of Health and Welfare, Canada, and the National Institutes of Health, Bethesda, Maryland. We are also indebted to the Charles E. Frosst Company and the Frank W. Horner Company, Montreal, and Eli Lilly and Company, Indianapolis, for financial assistance.

Received for publication Aug. 20, 1957.

*Ambrosia artemisiaefolia.

†The fractions in all the experiments were used on the basis of 1,000 units per cubic centimeter; 1 unit = 10^{-5} mg. nitrogen.

to absorption of the injected reagin and its redistribution, but was developed de novo by the subject. Were this reaginic activity due to absorbed reagin, one might expect that the same relative degree of activity to the WSR fractions would be exhibited by the allergic serum used in the passive transfer experiment and by the subject. However, the "acquired sensitivity" by the subjects was higher to $P_1 + C$ than to P_2, and was nonexistent to fractions $AA_1 + Pt$ and P_3 (except in subject M. D.), whereas the relative activity of the fractions

TABLE I. ACTIVITY OF WSR FRACTIONS BY PASSIVE TRANSFER

ALLERGIC SERUM USED	REACTION ELICITED BY FRACTION*					
	AA_2	AA_1 + PT	P_3	P_2	P_1 + C	WSR
B. K.	0	3+	4+	3+	2+	3+
B. L.	±	3+	4+	2+	2+	4+
B. P.	0	5+	6+	6±	3+	5+
J. S.	0	3+	4+	3+	1+	3+
B. W.	0	4+	5+	4+	2+	4+

*All fractions are made up to 1,000 units per cubic centimeter; 1 unit = 10^{-5} mg. nitrogen.

to allergic serum was in the order of P_3, $AA_1 + Pt$, P_2, and $P_1 + C$. Furthermore, it is interesting to note that the sera of two subjects (M. D. and A. L.) were capable of passively transferring their reaginic activity and that the patterns of the Prausnitz-Küstner reactions were identical to those observed on intradermal injection of the subjects. The sera of the other subjects were not tested by passive transfer. It is of interest, however, that none of these persons complained of having developed clinical hay fever or asthma during the following two years. One subject (M. D.) was skin-tested with the WSR fractions ten months after initially showing sensitivity, and the reactions were identical to those shown originally (as in Table II).

TABLE II

PERSON* TESTED	REACTION ELICITED BY FRACTION				
	AA_1 + PT	P_3	P_2	P_1 + C	WSR
D. K.	–	–	1+	3+	3+
M. D.	–	2+	3+	4+	3+
A. S.-B.	–	–	1+	3+	2+
A. L.	–	–	2+	3+	3+
R. H.	–	±	1+	2+	2+

*Clinically nonallergic person.

The rapidity with which this newly acquired sensitivity was developed by these subjects is most striking. In the case of one subject (R. H.) the sensitivity was noted only two days after the initial injection of the allergen fractions, while in the other subjects the sensitivity was demonstrated within three to four days. This is certainly a more rapid "primary" response than has ever been reported.[7] None of these persons had been injected previously with ragweed pollen or with any other pollen. However, all had been subject to airborne ragweed pollen which, as is known, can be absorbed through the nasal mucous membrane, the respiratory tract, and the digestive system.[3-6] Under

these circumstances, these persons could have become "subclinically" sensitized to the WSR components. Therefore, the possibility of our having evoked an anamnestic reaction in these subjects cannot be ruled out.

CONCLUSIONS

1. Some normal persons are capable of forming reagins in response to injections of allergens without becoming clinically allergic.

2. The reagins in these persons are detected within a surprisingly short interval of two to four days after initial injection of the allergens and can be passively transferred.

3. The WSR components which induced the formation of "reagins" in these persons appear to be different from those eliciting reagins in clinically allergic persons.

We should like to thank Dr. J. G. Harter for valuable comments and critical evaluation.

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EOSINOPHILIC PNEUMONITIS COMPLICATING AND MIMICKING PULMONARY TUBERCULOSIS

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EOSINOPHILIC pneumonitis, pulmonary eosinophilia, and Löffler's syndrome are terms used to describe the entity of pulmonary infiltration associated with blood eosinophilia. This condition was first brought sharply into focus in 1932 by Löffler,[1] who described it in his report of five cases. Since that time this combination of findings has frequently been referred to as Löffler's syndrome.

It has been known that eosinophilia is associated with many types of pulmonic involvement caused by a number of apparently unrelated diseases. This has been seen in the resolving stage of pneumonia, hydatid disease of the lung, Hodgkin's disease, and sarcoidosis. Pollinosis and parasitic disease of the lung have been described as etiological factors. Tropical eosinophilia has been reported as caused by a mite infestation of the lung, from whence this parasite has been recovered. Following the administration of penicillin, sulfonamides, and beeswax, pneumonia associated with eosinophilia has been seen and frequently accompanied by asthma. It has been produced by insufflation of egg albumin and injections of autogenous vaccine in sensitized animals and human beings, respectively. Leitner[2, 3] believed that some cases may be due to hypersensitivity to tuberculin. This disease has a wide range of clinical manifestations, from small evanescent pulmonary infiltrates to the most severe illness which may be evidenced by multiple organ involvement with polyarteritis.

In the excellent review by Crofton and associates,[4] it was felt that pulmonary eosinophilia might best be described as a condition in which pulmonary infiltration on x-ray is accompanied by blood eosinophilia but in which any of the four diseases (namely, resolving stage of pneumonia, hydatid cyst of the lung, Hodgkin's disease, and sarcoidosis) could be excluded. They went so far as to classify various stages of the disease and made the comment that pulmonary eosinophilia covers a wide range of diseases, varying from the mild and transient changes in true Löffler's syndrome to the severe and often fatal manifestations of polyarteritis nodosa with lung involvement.

Clinical findings are often very slight.[5] Cough may or may not be present. There often may be production of sputum which varies in character from mucoid to mucopurulent. Fever of low grade, seldom above 103 degrees, is usually present. The radiographic changes are more important than physical signs, which may be very hard to elicit or entirely absent. These x-ray changes are quite variable and usually consist of bilateral involvement with fleeting infiltrations which vary all the way from miliary or submiliary to large infiltrates, often

Received for publication Sept. 17, 1957.

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having the appearance of tuberculosis when involving the apices. Hilar adenopathy is frequently present. There may be involvement over the outer portions of the lung fields which in the posteroanterior view resemble the aftereffects of an explosion, with the appearance of smoke and dust curling up over the periphery of the lung fields. In one of our cases a similar condition was found, giving the appearance of cavitation which probably was due to emphysema. In the lateral views one may see various evidences of segmental involvement and platelike areas of atelectasis due to bronchial obstruction. The blood count usually varies from 6 to 60 per cent eosinophils and the total white count ranges from 10,000 to 70,000. Not infrequently there is involvement of other organs, with accompanying polyarteritis, sinus disease, pleural effusion, purpura, paralysis, adhesive pericarditis, pericardial effusion, urticaria, ulcerative colitis, enlarged hilar glands, encephalitis, and enlarged liver. As is noted, there may be extreme variability in the course of this disease, which may be mild and virtually without symptoms, lasting only a few days to a week or two, as contrasted to a very prolonged vitiating disease with involvement of various other organs and finally terminating in death.

Treatment has been diversified. Sulfonamides, penicillin, arsenicals, antihistamines, and finally ACTH have been used without, as a general rule, any striking benefit to the patient. The approach to the allergic problem where asthma has been present has been the most rewarding. In spite of the various measures taken in a certain group of seventy-eight patients,[4] nine died—three in an acute episode of asthma, several of cardiac failure, and one unexpectedly during administration of an Adrenalin spray. Except in one instance, all patients had other lesions in other organs besides the lungs.

Pathologic findings are nonspecific.[6] The acute pneumonitis has been characterized by edema, alveolitis, and bronchitis, and the perivascular lymphatics have been dilated, containing many erythrocytes. There have been occasional areas of acute arteritis and phlebitis with mural thrombosis. Harkavy[7] has stressed the presence of arterial infiltrations. Vascular lesions have been denied or their presence not described by other observers.

Certainly this disease is characterized by its protean nature. The cases presented here are additional proof of this. They are unique in that pseudocavitary involvement occurs in one of them and they both appear to represent instances of bacterial allergy. In neither case was the approach with simple antibiotic therapy satisfactory. Their successful outcome resulted from the combined use of steroid therapy and specific antibacterial treatment.

CASE REPORTS

CASE 1.—T. P., a 32-year-old Negro man, was hospitalized on Feb. 16, 1955, after a one-month illness consisting of productive cough, hoarseness, fever, 30-pound weight loss, anorexia, and easy fatigability. Past history revealed seasonal hay fever (ragweed pollinosis) since 1951. Physical findings showed signs of right upper lobe consolidation, mild hepatomegaly, and posterior cervical adenopathy. The patient appeared chronically ill, and his temperature was 102° F. X-ray studies (Fig. 1) revealed cavitary and infiltrative disease in the right lung field. Bacteriology was positive on smear and culture for tubercle bacilli.

The hemoglobin was 10.7 Gm.; the white blood cell count was 6,800 with 1 per cent eosinophils; and the serology and urinalysis were negative. Treatment with streptomycin (1 Gm. twice weekly) and isoniazid (100 mg. three times daily) was begun on Feb. 17, 1955, and the patient was afebrile by Feb. 20, 1955. There was rapid clinical improvement; sputum became negative on March 28, 1955, and progress x-ray films on April 13, 1955, showed 75 per cent clearing of his initial disease with evidence of cavity closure (Fig. 2). Ear, nose, and throat examination revealed early laryngitis, possibly tuberculous, and allergy studies confirmed a diagnosis of seasonal hay fever with strong direct skin test reaction to ragweed extract and slight sensitivity to feathers.

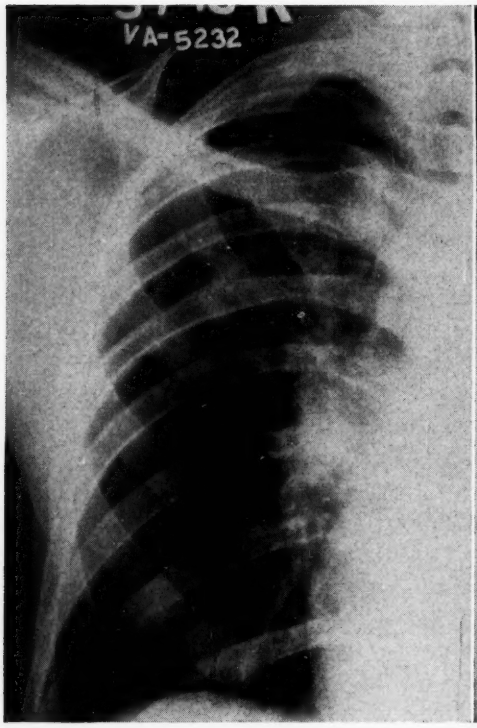

Fig. 1.

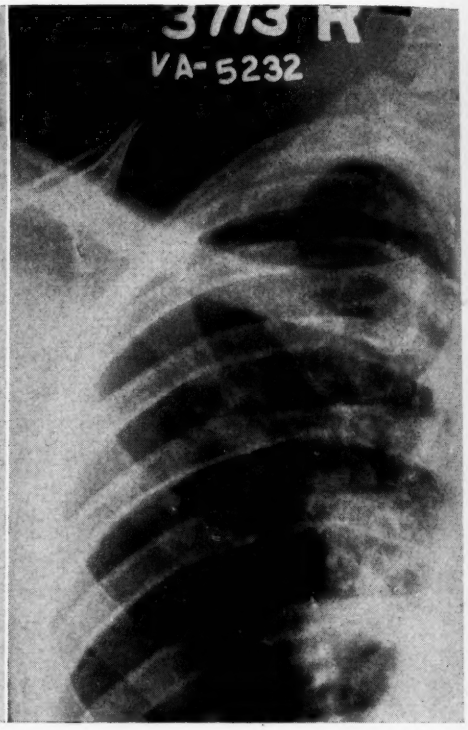

Fig. 2.

Fig. 1.—Case 1. Admission x-ray picture showing infiltrative disease in right upper lobe with a cavity overlying the second anterior rib.

Fig. 2.—Case 1. X-ray picture taken on April 12, 1955, showing clearing of the infiltrative disease. Cavity is now indistinct.

Beginning in May, 1955, during the fourth month of hospitalization, and continuing for the next five months, there were five separate episodes of pneumonia. Each was characterized by a gradual onset, chilly feelings, moderate to high fever, productive cough, substernal chest pain, and x-ray evidence of pneumonitis. During each recurrence, the leukocyte count remained within normal limits, with eosinophils varying between 4 and 11 per cent. Sputum studies again became positive for the tubercle bacillus and during each illness consistently revealed *Proteus vulgaris*. *Staphylococcus albus* and alpha hemolytic streptococcus were found intermittently. During the first episode, *Staphylococcus albus* was cultured from the blood stream.

During the first febrile period there was an increase in the right upper lobe disease. This was interpreted as a tuberculous spread, as the sputum had become positive during the

previous month. The finding of pyogenic organisms in the sputum prompted the use of tetracycline, with no apparent response. Penicillin was added, with rapid improvement, and x-ray evidence of resolution was noted. Blood cultures at this time were positive for *Staphylococcus albus;* penicillin was increased to 1.2 million units daily, and continued for six weeks. During the third week of therapy a mild febrile illness associated with a new area of infiltration appeared in the right upper lobe. This cleared on continued penicillin therapy. A more severe febrile episode appeared during the sixth week on penicillin and an area of consolidation appeared in the right medial base. Penicillin was discontinued, erythromycin was begun, and gradual clinical improvement and x-ray resolution occurred.

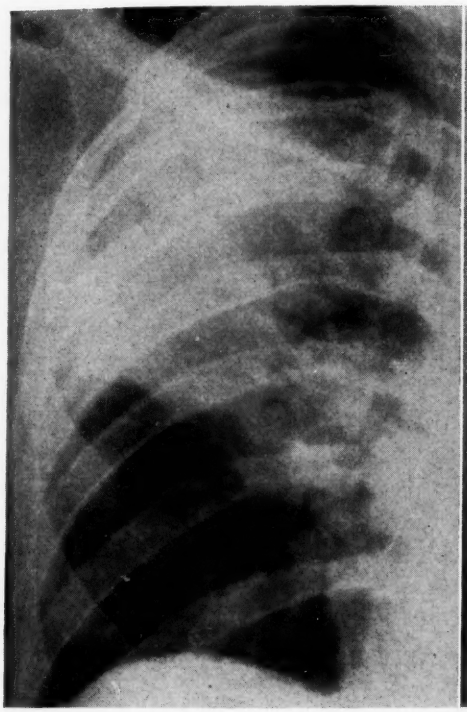

Fig. 3.

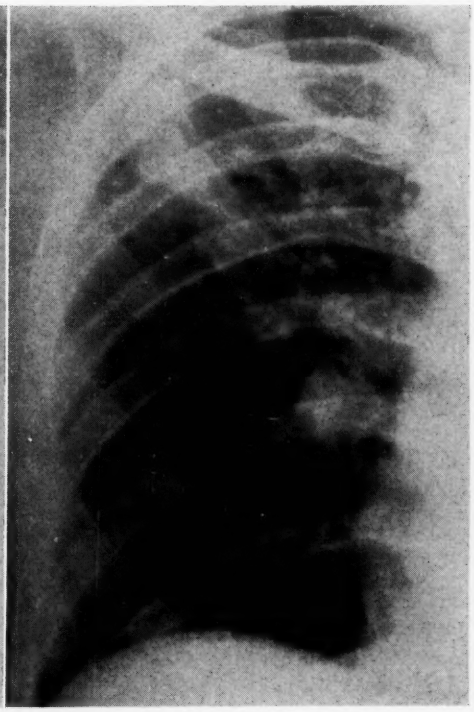

Fig. 4.

Fig. 3.—Case 1. X-ray picture taken on Oct. 10, 1955. Lobar consolidation has appeared in the right upper lobe. Hemoptysis occurred on Oct. 9, 1955, following bronchoscopy and this picture was felt to represent a combination of aspirated blood, tuberculous disease, and bacterial or Löffler's pneumonia.

Fig. 4.—Case 1. X-ray picture taken on Nov. 7, 1955. Progressive improvement had been noted since Oct. 21, 1955. Therapy with prednisone and oxytetracycline had now been given since Oct. 10, 1955. Following this film, no further x-ray improvement was noted until the date of surgery.

Erythromycin was continued, and during the second week of this therapy a low-grade fever with temperatures up to 101 degrees appeared. The patient now had severe vasomotor rhinitis. Antibiotic therapy was discontinued, except for the antituberculosis drugs (streptomycin and isoniazid); an antihistamine was begun and a hyposensitization procedure for ragweed pollinosis was started. The patient's temperature returned to normal prior to hyposensitization. No evidence of pneumonia was found at this point. One week later another febrile episode of greater severity occurred, with a temperature of 103° F., and an area of infiltration appeared in the left upper lung field. Penicillin and chloramphenicol were started. Serum

Fig. 6.

Fig. 5.

Figs. 5 and 6.—Case 2. Admission films taken March 30, 1955. Note bilateral disease with pseudocavitation on left and cardiomegaly.

proteins were 8.25 Gm. per cent, with 3.7 Gm. of globulin. Liver function was otherwise normal and plasma L.E. tests were negative. The sputum contained no eosinophils. The symptoms gradually subsided.

Following this, all medications were stopped to evaluate the possibility of drug sensitivity leading to Löffler's pneumonia. Five days later the fifth and most severe febrile episode occurred, associated with increased right upper lobe infiltration. No eosinophils were seen in the sputum. The patient's general condition again gradually improved on penicillin plus chloramphenicol and he became afebrile, despite x-ray evidence of incomplete resolution. During the afebrile period, the sputum was found to contain significant numbers of eosinophils. He was bronchoscoped on Oct. 9, 1955, as the more recent right upper lobe disease had not resolved. Bronchoscopic washings from the right upper lobe were sterile on culture and contained 60 per cent eosinophils. Sputum cultures during this time continued to grow *Proteus vulgaris,* alpha hemolytic streptococcus, and *Staphylococcus albus.* A massive hemoptysis occurred following bronchoscopy. The patient nearly exsanguinated, but was saved by emergency blood replacement and supportive measures. There were physical signs and x-ray evidence (Fig. 3) of lobar consolidation in the right upper lung. The temperature was 105° F. and the patient showed signs of circulatory failure. Prednisone, 60 mg. daily, was begun with oxytetracycline, 1 Gm. daily; isoniazid was resumed and viomycin was started. Rapid improvement ensued. X-ray pictures on Oct. 21, 1955, showed extensive resolution of the right upper lobe consolidation (Fig. 4). There were no further hemoptyses.

Further improvement continued under prednisone plus antibiotic therapy until Jan. 5, 1956, when the chest appeared to have stabilized. The patient had remained positive since May, 1955. A cavity was now present in the right upper lobe, along with infiltrative disease there and in the superior segment on the left. The patient's temperature thereafter remained within normal limits and no further episodes of fever or pneumonitis occurred.

During September, 1955, antibiotic therapy was modified when drug resistance was entertained as a possible cause of the recurring episodes of pneumonitis. PAS was added and was discontinued after one week. As drug fever from streptomycin was also considered possible, a one-month course of streptomycin hyposensitization was begun. Viomycin was started on October 13 and streptomycin was discontinued, as the febrile episodes continued. Beginning on Dec. 12, 1955, isoniazid resistance appeared and the patient was placed on streptomycin, PAS, isoniazid, and pyrazinamide. In view of the persistent cavity, persistence of positive sputum, and resistance to INH, a right upper lobectomy was performed on March 14, 1956. Pathologic study of the resected specimen showed all stages of tuberculous granulation tissue, but chiefly active with bronchial involvement and erosion. The final pathologic diagnosis was fibrocaseous tuberculosis with cavitation and bronchiectasis.

Case 2.—A. C., a 68-year-old white woman, was hospitalized on March 29, 1955, with the complaint of a productive, harassing cough and fever since February, 1955. Early in her illness she developed considerable pain posteriorly in the right shoulder and became progressively more dyspneic, with considerable embarrassment on coughing and even on talking. Easy fatigue had been present since the onset of her illness, and she had experienced pain in her chest when coughing and on bending. Past history revealed that the patient had had mild asthma for years and had undergone a nasal polypectomy. She was initially hospitalized and treated with tetracycline for one week, after which she was transferred to a tuberculosis sanatorium where she remained for three and one-half weeks before it was decided that she did not have tuberculosis.

Physical examination revealed the patient to be a well-developed, slightly overnourished, florid, 68-year-old white woman. The blood pressure was 130/80. She appeared flushed and febrile. There was purulent rhinitis. The posterior cervical triangle lymph nodes were palpable. There was retraction with decreased expansion of the left chest. A pleural friction rub was heard along the left margin of the sternum and close to the midline in the back. Subcrepitant râles were heard in both lungs, with more on the left than on the right. An occasional inspiratory rhonchus was heard in both lung fields. The spleen was not felt. Examination of the heart revealed P_2 to be louder than A_2 and there was a suggestion that the heart was somewhat enlarged.

X-rays revealed a pansinusitis with membrane thickening of both antra. Chest x-rays showed the heart to be enlarged in its transverse diameter. Scattered densities were seen in both upper lung fields and behind the heart. A cavity was seen in the left upper lobe over the third rib (Figs. 5 and 6). The considerations were tuberculosis, bronchiectasis with cavity, staphylococcus pneumonia, collagen disease, and Boeck's sarcoid.

Planigrams of the upper lobe taken on April 1, 1955, revealed an emphysematous bleb of the left upper lobe (Fig. 7). The Wassermann reaction was negative and an electrocardiogram showed minor changes felt to be consistent with myocardial changes on a coronary sclerotic basis. A urinalysis showed a slight trace of albumin, but was otherwise unremarkable. There were 11.6 Gm. hemoglobin, 15,000 white blood cells, 72 per cent segmented cells, 5 per cent band cells, 7 per cent eosinophils, 13 per cent lymphocytes, and 3 per cent monocytes. The hematocrit was 37 per cent; the sedimentation rate was 16 mm. fall in fifteen minutes, 49 mm. in thirty minutes, and 95 mm. in sixty minutes. Toxic granules were noted in the polymorphonuclear leukocytes. The heterophile antibody test was negative. The albumin-globulin ratio was 3.7/2.8. The fasting blood sugar was 130 mg. and the nonprotein nitrogen was 35 mg. per cent. The C-reactive protein was 8 units. Sputum examination showed normal flora repeatedly and no fungi were recovered. The febrile agglutinins were zero. Histoplasmin and coccidioidin skin tests were negative. Bone marrow was normal. The alkaline phosphatase was 8.5 King-Armstrong units and the serum calcium was 9.5 mg. per cent. Cultures were later reported negative for tubercle bacilli.

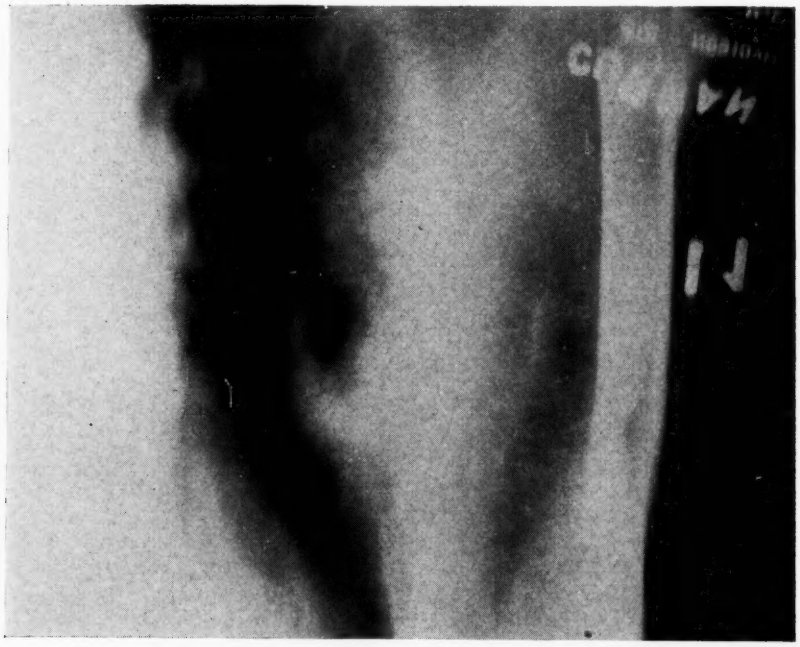

Fig. 7.—Case 2. Planigram taken April 1, 1955, showing bleb formation best seen at 11 cm. level.

A first-strength tuberculin test on April 3, 1955, was negative. The second strength was also negative at forty-eight hours. The tuberculin test was repeated on April 11, 1955, as it was thought that there was a considerable delayed reaction to the second test appearing at ninety-six hours. The repeat second-strength test appeared to elicit a 3 plus reaction after twenty-four hours; this became very intense by thirty-six hours, with a threatened slough and a rash rapidly migrated in centrifugal fashion to involve the entire body in an erythema multiforme type of reaction. Repeated blood counts showed up to 11 per cent eosinophils.

Since, in the early phase of the illness, the patient was treated with tetracycline alone without response, hydrocortisone was added at a dose of 240 mg. per day. The tetracycline was begun again in the amount of 1.5 Gm. per day and was given for the next two weeks, when it was replaced by erythromycin in the same dose. The patient rapidly improved and became afebrile after a few days. The chest signs and rash cleared consistently and, at the time of her discharge on April 23, 1955, she was free of symptoms. She was discharged on erythromycin and 80 mg. of compound F daily.

The patient has been followed for one year and has finally been taken off the steroids and antibiotics. Her chest has remained clear roentgenographically (Fig. 8), and the only residual of this pneumonic process is a wedge-shaped area of atelectasis above the left diaphragm.

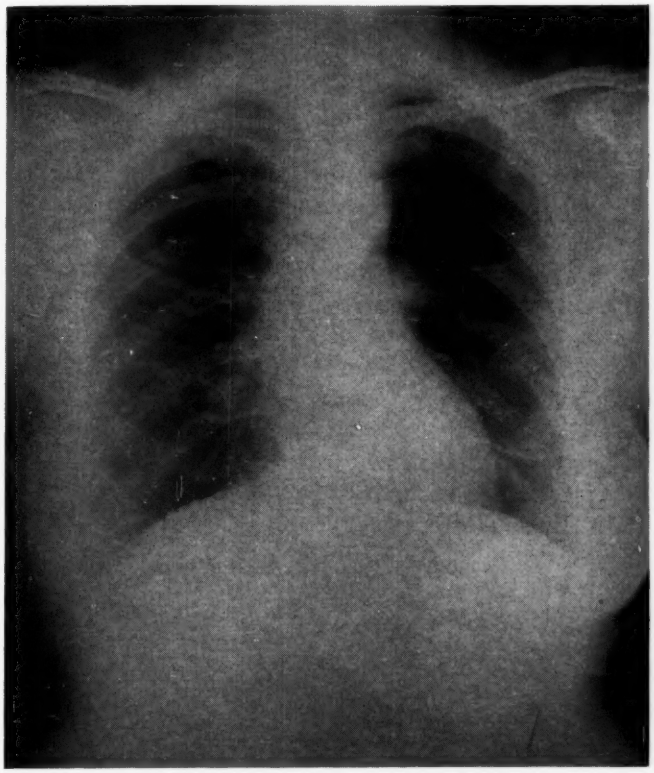

Fig. 8.—Case 2. X-ray picture taken one year later showing residual area of atelectasis above left diaphragm.

DISCUSSION

Certainly this disease is characterized by its extreme variability. The cases just presented offer additional proof of this. They are unique in that pseudocavitary involvement occurred in one of them and they both appear to represent instances of bacterial allergy. In neither case was the approach with simple antibiotic therapy alone effective. Their successful outcome was due to the combined use of steroid therapy and specific antibacterial treatment.

In Case 1 a tuberculous pneumonia responded rapidly to appropriate antibacterial treatment, only to relapse in the face of such treatment even when the

tubercle bacillus was still sensitive to the same medication. Complicating heterologous bacterial pathogens were found; the sputum remained positive for the tubercle bacillus; blood eosinophilia was present and eosinophil-laden mucus was aspirated from the tracheobronchial tree. It is perfectly possible that one or two types of bacterial sensitivity operated. One possibility is that the pneumonitis was simply due to a sensitivity reaction to the nontuberculous respiratory bacterial pathogens. The other possibility is that, in the face of hypersensitive necrotizing pneumonia due to the tubercle bacillus, there was simply aggravation of the tuberculous process by the toxins from the heterologous organisms. An example of this nonspecific type of reaction is seen in the Shwartzman phenomenon, in which a typical response is obtained in animals prepared by injecting one type of bacterial toxin intradermally and then challenging them intravenously in twenty-four hours by toxins derived from heterologous organisms.

In the first case, the aggravating influence of pollinosis undoubtedly operated, although the span of time during which the episodes of recurring pneumonitis occurred considerably antedated and followed the ragweed season in Wisconsin. As will be noted, the acute phase of this illness lasted from May through October.

Case 2 was especially interesting in that the patient exhibited pseudocavitary disease along with eosinophilia. There was evidence of multiple serous membrane involvement and it was felt that initially she was suffering from pulmonary tuberculosis. A trial course of antibiotics did not help her and her symptoms progressed until a steroid was added to her treatment. The case is of further interest in that there was apparently sensitization to tuberculin on repeated testing which resulted in a generalized tuberculid. This also may be an example of challenge with reaction by a heterologous toxin in a patient already sensitized by a bacterial toxin.

SUMMARY

1. The two cases of classical Löffler's syndrome characterized by pulmonary infiltration and blood eosinophilia presented seemingly represent bacterial hypersensitivity. In one instance, the eosinophilic pneumonitis accompanied pulmonary tuberculosis and in the other it resembled tuberculosis.

2. Effective treatment required appropriate antibiotics and steroid therapy in combination.

3. Aspects of the syndrome of pneumonia and eosinophilia have been discussed and their possible mechanisms have been described.

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STAPHYLOCOCCUS AUREUS ANTIGENS IN HYPERSENSITIVITY REACTIONS AND EXPERIMENTAL ARTERIAL SENSITIZATION

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WITH THE TECHNICAL ASSISTANCE OF VINCENT A. J. DRAPIEWSKI****

INTRODUCTION

INVESTIGATION into the subject of bacterial sensitivity has been hampered by the physical state of the bacterial cell itself participating in in vivo antigen-antibody reactions. It is always difficult to evaluate the role of an intact cell as a true antigen in studying hypersensitivity phenomena, as its particulate nature and cellular function might suggest a biologic effect apart from a suspected role in immunologic reactions. Then, too, biochemical laboratory procedures desgined to fractionate the living cell into protein, carbohydrate, and lipid components entail the risk of creating new compounds that might result from the splitting and/or rearrangement of the labile, complex, molecular aggregates that may have been characteristic of intracellular structure.[1] The report of Dworetzky and co-workers[2] has demonstrated the physiologic and immunologic action of *Staphylococcus aureus* preparations obtained by grinding live microorganisms in a ball mill, and our studies[3] have demonstrated the retained antigenicity of *Staphylococcus aureus* cytoplasm recovered from the disruption of live bacterial cells when exposed to ultrasonic vibrations. This availability of antigenic bacterial cytoplasm has furnished a convenient tool for the further study and consideration of bacterial hypersensitivity for in a soluble state biologically active *Staphylococcus aureus* bacterial preparations may lend themselves to participation in such attempted in vivo antigen-antibody reactions as anaphylactic shock, Arthus cutaneous reactions, and experimentally induced lesions of vascular sensitization in experimental animals.

From the Department of Biology, Wilkes College.

Presented in part at the thirteenth annual meeting of the American Academy of Allergy, Los Angeles, California, Feb. 4-6, 1957.

This investigation was supported (in part) by a research grant (No. H-2412) from the National Heart Institute, Public Health Service.

Soluble bacterial cell protoplasm employing ultrasonic vibrations was prepared under the direction of Dr. Sam Beiser, Department of Microbiology, Columbia University, College of Physicians and Surgeons, New York.

Quantities of *Staphylococcus aureus* live bacterial organisms in a frozen and dried state and staphylococcus toxoid were kindly supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, through the courtesy of Dr. H. D. Piersma.

Received for publication April 15, 1957.

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**Research Assistant.

***Assistant Professor of Biology.

****Student Laboratory Assistant.

EXPERIMENTAL PROCEDURES AND RESULTS

Antigens.—All bacterial antigens used for immunization and challenging procedures were derived from broth cultures of the same strain of *Staphylococcus aureus,* Wood 46.

1. *Vaccine:* Saline suspension of phenol killed whole bacterial cells.

2. *Undenatured cellular protoplasm:* Prepared by subjecting saline suspensions of whole live bacterial cells to the effects of ultrasonic waves at 17,000 cycles per second. Bacterial debris consisting chiefly of cell walls was separated from the soluble cellular contents by centrifugation and Seitz filtration. The resultant sterile filtrate contained 1 mg. nitrogen per milliliter.

3. *Toxoid:* A culture filtrate of *Staphylococcus aureus* treated by pepsin digestion and containing 10,000 dermonecrotizing units per milliliter.

Experimental Animals.—Male rabbits varying between 2.5 and 3.5 Kg. in weight were used throughout in active immunization procedures for the production of antisera and for all passive sensitization and challenge experiments.

Antisera.—

1. *Antibacterial sera:* This was prepared by immunizing rabbits with intravenous and intradermal injections of vaccine for a total dose of 21 billion bacterial organisms administered in divided doses over a twenty-one-day period. Seven days after the last injection the rabbits were bled by cardiac puncture; the sera were pooled and sterilized by Seitz filtration. The antiserum was capable of agglutinating *Staphylococcus aureus* bacterial cells in in vitro macroscopic tests in a serum titer of 1:2,560 and formed interfacial precipitate when overlayed in test tubes with saline dilutions of *Staphylococcus aureus* soluble cytoplasm and toxoid.

2. *Antitoxin:* This was prepared by immunizing rabbits with intramuscular injections of toxoid adsorbed on aluminum hydroxide cream adjuvant at multiple sites for a total dose of 100,000 dermonecrotizing units. Twenty-one days later the animals were bled by cardiac puncture; the sera were pooled and sterilized by Seitz filtration. This antiserum was capable of agglutinating *Staphylococcus aureus* bacterial cells in in vitro macroscopic tests in a serum titer of 1:80 and formed interfacial precipitate when overlayed in test tubes with saline dilutions of *Staphylococcus aureus* soluble cytoplasm and toxoid.

Anaphylaxis.—Anaphylaxis was uniformly produced in normal rabbits following passive sensitization and challenge experiments with *Staphylococcus aureus* antisera-antigen systems. Passive sensitization was accomplished by the intravenous administration of 5 ml. of homologous species antisera capable of agglutinating *Staphylococcus aureus* bacterial cells in a serum titer of 1:2,560.

Thirty minutes later they were challenged with a soluble preparation of *Staphylococcus aureus* cellular protoplasm. Within 2 to 3 minutes after receiving the minimal critical dose of 0.25 mg. N, typical signs and symptoms of fatal anaphylactic shock appeared with the resultant gross pathologic finding of right heart dilatation. Abnormal signs and symptoms were completely absent in the control series of animals receiving either the 5 ml. of antiserum or 0.25 mg. N of soluble bacterial cell protoplasm alone.

Cutaneous Reactions.—The intradermal injection of saline dilutions of *Staphylococcus aureus* soluble cell protoplasm preparations in normal rabbits resulted in the development of cutaneous lesions of erythema, edema, and induration over a twenty-four to forty-eight-hour period at the site of injection and usually progressed to resemble the dermonecrotic lesions observed in rabbits following the intradermal injection of *Staphylococcus aureus* culture filtrate or toxoid. The development of these skin lesions following antigen injections alone prevented the study and evaluation of the ability of *Staphylococcus aureus* antigen-antibody systems to participate in in vivo cutaneous hypersensitivity reactions of the Arthus reaction type in sensitized rabbits prepared for challenge by the intradermal route. Cutaneous reactions failed to develop in rabbits in whom local skin sites were prepared by intracutaneous injections of antibacterial agglutinating antiserum or precipitating antitoxin and subsequently challenged with the intravenous administration of soluble bacterial cell protoplasm and toxoid, respectively.

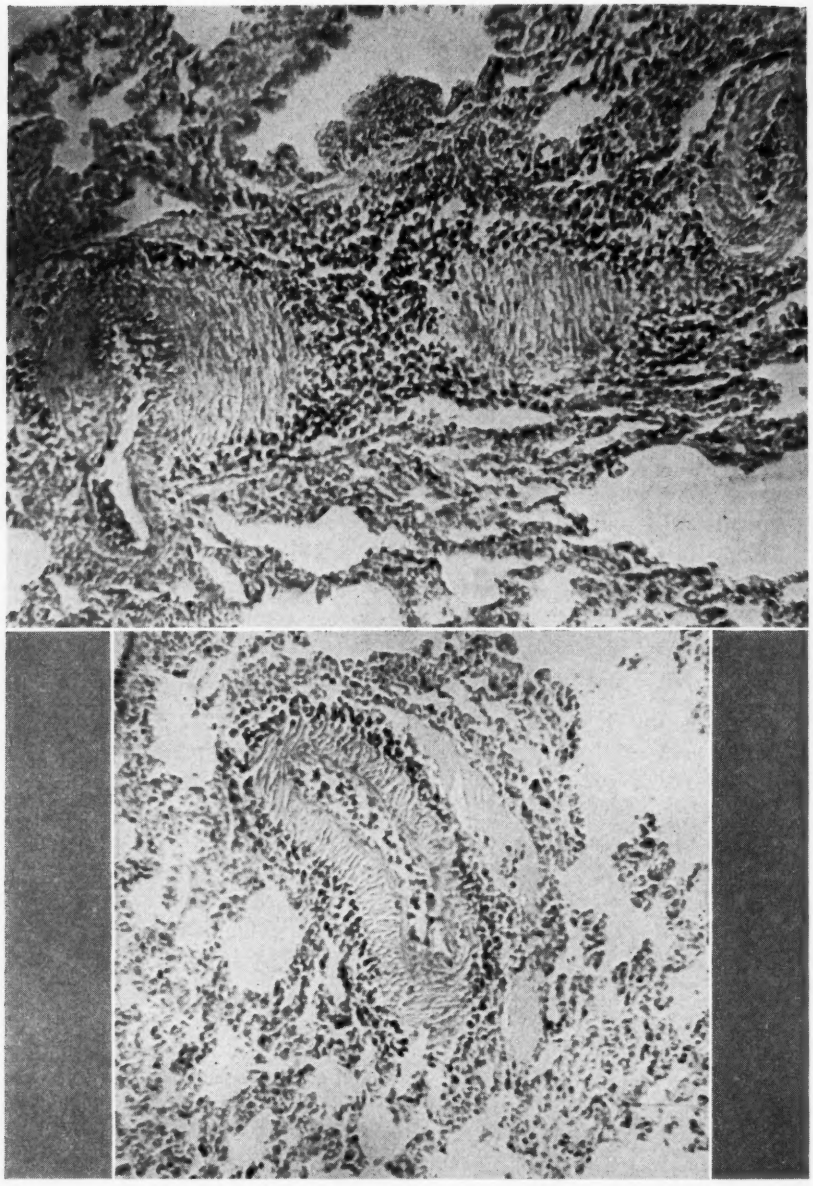
Arteritis.—The techniques previously described, favorable for the production of lesions of pulmonary periarteritis and panarteritis with horse serum-anti-horse serum[4] and streptococcus toxin-antitoxin[5] systems, were extended to evaluate potential vascular sensitizing properties of *Staphylococcus aureus* antigen-antibody systems. In this procedure normal animals are passively sensitized with the intravenous administration of a high-titer antiserum. After an incubation period of thirty minutes the subsequent intravenous challenge

TABLE I. THE NUMBER OF RABBITS DEVELOPING LESIONS OF PULMONARY PERIARTERITIS AND PANARTERITIS FOLLOWING PASSIVE SENSITIZATION WITH HOMOLOGOUS SPECIES RABBIT AGGLUTINATING ANTISERA VERUS STAPHYLOCOCCUS AUREUS BACTERIAL CELLS OR ANTITOXIN AND SUBSEQUENTLY CHALLENGED WITH SOLUBLE CELL PROTOPLASM OR TOXOID OF THE SAME BACTERIAL SPECIES

NO. OF ANIMALS	SENSITIZING ANTISERUM VERUS STAPHYLOCOCCUS AUREUS	CHALLENGING ANTIGEN STAPHYLOCOCCUS AUREUS	NO. OF ANIMALS WITH LESIONS
6	5 ml. antiserum for whole cell	Cell protoplasm 0.25 mg. N	5
6	5 ml. antiserum for whole cell	———	0
6	———	Cell protoplasm 0.25 mg. N	0
5	5 ml. antiserum for whole cell	Toxoid 5,000 dermonecrotizing units	4
5	5 ml. antitoxin	Cell protoplasm 0.25 mg. N	4
5	5 ml. antitoxin	Toxoid 5,000 dermonecrotizing units	4
6	———	Toxoid 5,000 dermonecrotizing units	0

The control series of animals received only the antisera or preparations of the bacterial antigens.

A.



B.

Fig. 1.—Sections of lung tissue from rabbits that had been passively sensitized with homologous species *Staphylococcus aureus antisera* and subsequently challenged with the intravenous administration of subanaphylactic doses of soluble *Staphylococcus aureus* antigens. The involved arteries and arterioles show marked perivascular and adventitial infiltration of eosinophils and some polymorphonuclear leukocytes and, to a lesser extent, of a few lymphocytes. Some intimal reaction can be seen in *A* and *C*. Eosinophils can be seen penetrating the media and intima in *E*. Collections of eosinophils are found within the lumen of vessels seen in *B* and *C*, and early thrombus formation has occurred within the lumen of one vessel seen in *B*. High-power magnifications of sections *E* and *F* demonstrate the eosinophilic cellular perivascular and adventitial infiltrates.

(Fig. 1, C to G on opposite page.)

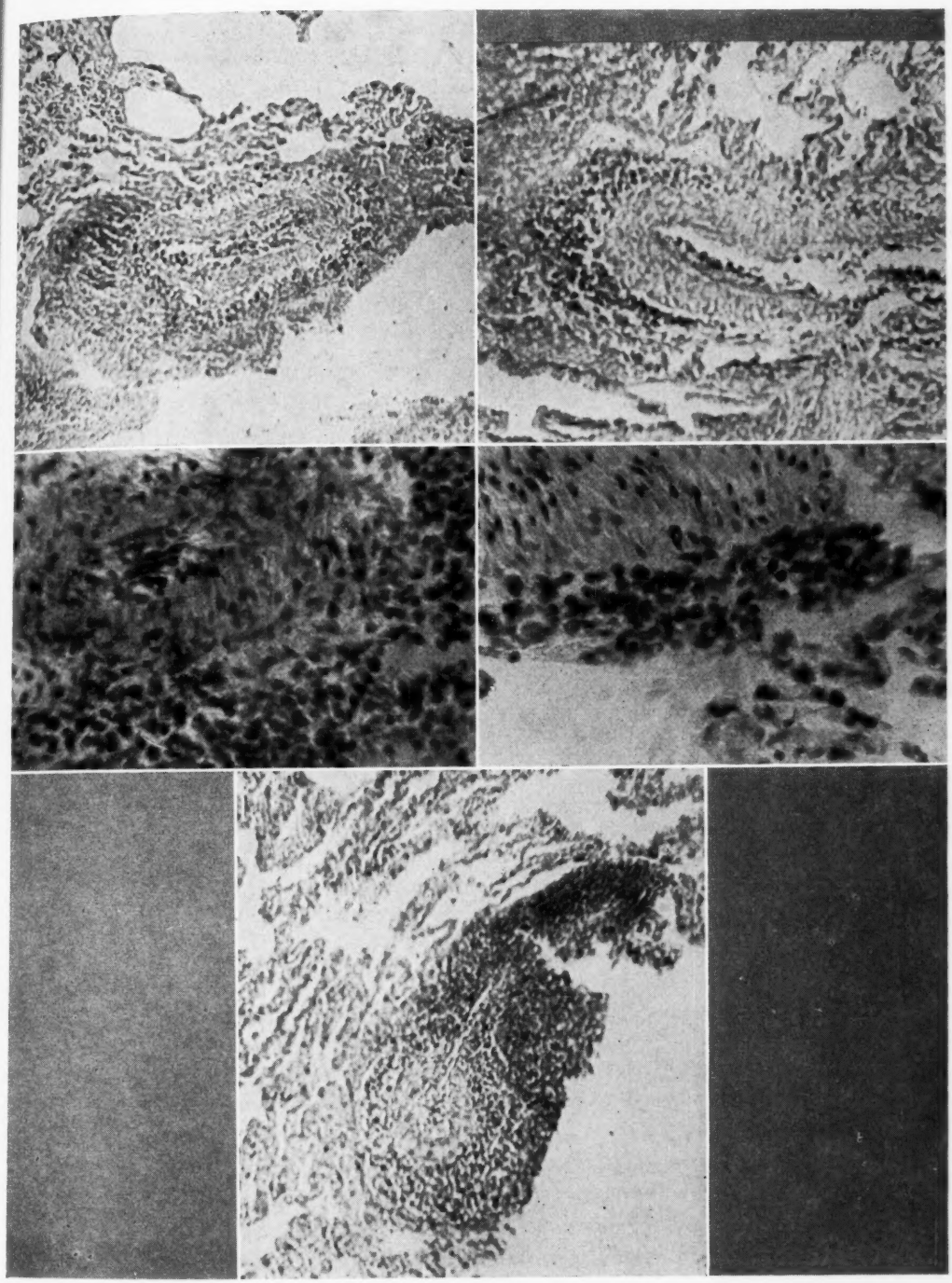


Fig. 1.—*C* to *G*. *(For legend, see opposite page.)*

of these animals with the soluble specific antigen is started. A total dosage of antigen previously determined to be the minimal critical dose capable of producing fatal anaphylactic shock is divided into four fractional dosages, each given at fifteen-minute intervals in graded increasing concentrations over the period of one hour in an attempt to effect an asymptomatic subacute anaphylactic reaction. Twenty-four hours later the animals are sacrificed by air embolism; sections are taken from the internal organs, prepared, and stained for histologic study.

The *Staphylococcus aureus* heterologous species rabbit antisera used for passive sensitization consisted of 5 ml. of either an antiserum for the whole cell with a bacterial agglutinating titer of 1:2,560 or a precipitating antixoin. The *Staphylococcus aureus* antigens used in challenging the passively sensitized rabbits consisted of either: (1) soluble bacterial cell protoplasm in divided doses of 0.025, 0.05, 0.75, and 0.1 mg. N, or (2) toxoid in divided doses of 500, 1,000, 1,500 and 2,000 dermonecrotizing units. The results are summarized in Table I.

Passive sensitization with antisera for the whole bacterial cell: When challenged with soluble cell protoplasm, five out of six rabbits showed pulmonary vascular lesions. When the animals were challenged with toxoid, pulmonary vascular lesions were demonstrated in four out of five rabbits.

Passive sensitization with antitoxin: When challenged with toxoid, four out of five rabbits showed pulmonary vascular lesions. When the challenge was made with soluble cell protoplasm, pulmonary vascular lesions were demonstrated in four out of five animals.

Lesions of the pulmonary vasculature could not be found in six rabbits receiving only antiserum for the whole cell, five rabbits receiving antitoxin, six rabbits receiving only soluble cell protoplasm, and six rabbits receiving toxoid alone.

In those series of animals where lesions were demonstrated following passive sensitization and challenge experiments, the pulmonary vasculature was found to be chiefly involved (Fig. 1). The small pulmonary arteries and pulmonary arterioles showed marked perivascular and adventitial infiltration of eosinophils and, to a lesser extent of polymorphonuclear leukocytes and some lymphocytes. Eosinophils were also found infiltrating the intima and media of the small arteries and arterioles, and in several instances collections of eosinophils and early thrombus formation were found within the lumina of these vessels. In several sections varying degrees of hyperplasia of the endothelial cells of the intima, cloudy swelling of the muscle fibers with hydropic degeneration of the media, and some adventitial edema were found. Scattered and light interstitial infiltration with some eosinophils was seen occasionally within some alveolar walls.

DISCUSSION

Typical symptoms and gross pathologic findings of anaphylactic shock in rabbits were uniformly elicited in animals passively sensitized by the intravenous

administration of homologous species *Staphylococcus aureus* antibacterial agglutinating sera and subsequently challenged with the soluble cellular protoplasmic contents of the same bacterial strain. Bacterial cells have previously been shown capable of causing anaphylactoid reactions in sensitized animals, but the role of the cells as antigen in these instances has been difficult to evaluate because of the toxicity of the bacterial preparations, per se, and because of their particulate nature. Thus, it has not been possible to rule out their role as intact particles in possible thrombus formation and/or embolization.[6] However, the reduction of cells to undenatured cellular antigen by the gentle but effective disrupting action of ultrasonic waves on the cell wall and the employment of this soluble material as the challenging antigen would tend to avoid such complicating factors and offer evidence to suggest a true sensitizing role for *Staphylococcus aureus* cytoplasm in such in vivo reactions of hypersensitivity as anaphylaxis. Little need be said regarding the value of the established technique of passive sensitization and challenge in allowing for the controlled evaluation of in vivo effects of the action of either antibacterial serum or the *Staphylococcus aureus* antigen. This further lends itself to the establishment of a minimal critical dose of challenging antigen prerequisite for anaphylaxis. In this regard, its value in circumventing the interfering factor of possible anaphylactoid reactions in nonsensitized animals resulting from toxic effects of larger doses of *Staphylococcus aureus* bacterial antigen is evident.

It was impossible to evaluate any potential for localized skin-sensitizing effects of intracutaneously administered soluble bacterial cell protoplasm because of the local cutaneous reactions of erythema, edema, induration, and progression to necrosis that resulted from the local injection of this material in dosages considered prerequisite for interreaction with specific antibody-containing homologous species serum for the production of local Arthus reactions. Kahn[7] has noted similar inflammatory type "flare-up" reactions in rabbits injected intradermally with killed suspensions of staphylococcus and attributed these to the natural immunity possessed by this animal to these microorganisms. However, the results of agar gel diffusion plates in an attempt to resolve *Staphylococcus aureus* cytoplasm into constituent antigens would suggest that the dermonecrotizing effects observed may be partially due to the actual exotoxin content demonstrated within the bacterial cell iself in the particular strain of this microorganism studied and reported here.[3]

Previous studies[4, 5, 8-10] employing the technique of passive sensitization and challenge have demonstrated that pulmonary lesions of periarteritis and panarteritis in the rabbit arise as a result of in vivo antibody-antigen reaction and probably as a tissue manifestation of subacute anaphylaxis. The results of the experimental procedure concerned with *Staphylococcus aureus* antibody-antigen systems reported here would tend to offer further evidence in this regard. These lesions may arise as a result of hypersensitivity reactions involving either the cellular cytoplasm and/or the exotoxin of *Staphylococcus aureus* and do not differ significantly in histologic detail from those resulting from the action of other protein antigen-antibody systems, such as horse serum-antihorse

serum. Their development, therefore, probably depends upon a tissue response to reactions of hypersensitivity rather than to any particular specific characteristic of the nature of the bacterial antigen or antiserum employed.

In vitro cross reactions in macroscopic agglutination and precipitin tests and precipitin band reactions of identity in agar gel diffusion plates have been observed between various *Staphylococcus aureus* cellular protoplasmic and toxoid antigens when tested against their respective antisera.[3] These probably arise as a result of each sharing some immunologically similar or identical component antigens. The fact that tissue lesions of vascular sensitization can arise in rabbits after challenge with either staphylococcus cellular protoplasm or toxoid, regardless of whether the animal has been passively sensitized to the bacterial antisera or to the antitoxin, would point out the possibility of similar cross reactions occurring in vivo, as well as in in vitro test procedures. Ehrich[11] has observed the development of primary mesenchymal reactions in rabbits following the intravenous injection of whole bacteria. However, the control studies utilizing only preparations of cell protoplasm, toxoid, antibacterial serum, or antitoxin suggest that lesions of pulmonary periarteritis and panarteritis described here arise as a result of tissue response to *Staphylococcus aureus* antibody-antigen reactions apart from any other tissue effect, toxic or otherwise, that may possibly be exerted by *Staphylococcus aureus* antigens or the presence of their antisera.

SUMMARY

1. The sensitizing properties of *Staphylococcus aureus* (Wood 46 strain) were studied, utilizing soluble cell protoplasm obtained by exposing live bacterial cells to the disrupting effects of ultrasonic vibrations and pepsin-digested culture filtrate (toxoid) as antigens.

2. In rabbits passively sensitized with high-titer bacterial agglutinating antisera, fatal anaphylaxis could be uniformly elicited by subsequent intravenous challenge with a soluble preparation of bacterial cytoplasm.

3. When the previously determined critical anaphylactic dose of soluble bacterial cellular protoplasm was administered intravenously in fractional dosages over a sixty-minute time period to rabbits passively sensitized with the same high-titer bacterial agglutinating antiserum, lesions of pulmonary periarteritis and panarteritis were demonstrated twenty-four hours later.

4. Lesions of pulmonary panarteritis and periarteritis were also observed when similar techniques were employed in rabbits passively sensitized with high-titer bacterial agglutinating antiserum and challenged with toxoid and in rabbits passively sensitized with antitoxin and challenged with either toxoid or soluble bacterial cellular protoplasm.

5. Neither anaphylactoid reactions nor the development of lesions of pulmonary periarteritis or panarteritis were observed in normal rabbits receiving only the same bacterial agglutinating antisera, antitoxin, soluble bacterial protoplasm, or toxoid.

6. The intracutaneous injection of preparations of soluble bacterial cellular protoplasm in normal nonsensitized rabbits resulted in the development of cutaneous lesions of erythema, edema, infiltration, and necrosis. This resembled the dermonecrotizing action of *Staphylococcus aureus* toxin and may have been due to the antigenic exotoxin components previously demonstrated within *Staphylococcus aureus* bacterial cells.

7. The sensitizing properties of the cytoplasm and toxin of *Staphylococcus aureus*, their antigenic relationships, and their ability to participate in such in vivo reactions of hypersensitivity in the rabbit as anaphylaxis and the development of tissue manifestations of vascular sensitization have been demonstrated.

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FAILURE TO INDUCE ALLERGIC RESPONSE IN GUINEA PIGS WITH SCORPION VENOM

DIANE S. DORO, EDWARD S. ORNELAS, AND ROY M. JOHNSON, TEMPE, ARIZ.

SNAKE venoms have been utilized as therapeutic agents in arthritic patients.[1, 2] The use of venoms as therapeutic agents necessitates a consideration of their allergic response. The venom of *Centruroides sculpturatus*, a poisonous scorpion indigenous to the southwestern United States, was tested for its ability to incite an allergic response in guinea pigs.

MATERIALS AND METHODS

The venom was obtained by electrical stimulation of the scorpion, lyophilized in vials, and weighed dry. One vial was used for the anaphylaxis experiments, another for the Schultz-Dale tests. The dried venom was dissolved in normal saline to give a concentration of 2 mg. per milliliter. This was used as a stock solution from which working dilutions in normal saline were made. The minimum lethal dose (M.L.D.) for each vial was determined by guinea pig titration, using eight male guinea pigs weighing between 500 and 900 grams each. All injections were made subcutaneously.

Four male guinea pigs weighing between 600 and 800 grams were inoculated with a sublethal concentration of venom for both the initial dose and, fourteen days later, the challenging dose (Table I).

TABLE I. ANAPHYLAXIS EXPERIMENT WITH C. SCULPTURATUS VENOM IN GUINEA PIGS*

M.L.D. VENOM (MG./KG.)	INITIAL INJECTION (MG./KG.)	SHOCKING DOSE (MG./KG.)	ANAPHYLAXIS
0.06	0.04	0.04	(−)

*All figures are in terms of milligrams of venom per kilogram of body weight.

Three male guinea pigs, each weighing between 600 and 800 grams, were injected with sublethal amounts of venom according to the schedule in Table II. The guinea pigs were subsequently sacrificed; the intestinal tissue was placed in Ringer's solution and the Schultz-Dale[3] reaction was attempted immediately.

The intestinal tissue was fastened to the bottom of a vial containing approximately 10 ml. Ringer's solution. The free end was attached to a lever arm in contact with a kymograph. The venom, in a concentration of 0.1 mg. per milliliter, was added dropwise to the exposed tissue by means of a tuberculin syringe and a 20-gauge needle.

From the Poisonous Animals Research Laboratory, Arizona State College.
Received for publication May 13, 1957.

RESULTS

In no experiment was any allergic reaction noted. Blood counts taken at the time of the Schultz-Dale experiments indicated no eosinophilia.

TABLE II. SCHULTZ-DALE EXPERIMENT WITH C. SCULPTURATUS VENOM*

GUINEA PIG NO.	M.L.D. VENOM (MG./KG.)	INITIAL INJECTION (MG./KG.)	SUBSEQUENT INJECTIONS	ANIMAL SACRIFICED (DAYS)	REACTIONS
1	0.1	0.08	None	15	None
2		0.08	0.08 on second, seventh, and tenth days	15	None
3		0.08	8 injections in 29 days, increasing venom concentration by 0.02 units to 0.14	45	None

*All figures are in terms of milligrams of venom per kilogram of body weight.

DISCUSSION

The use of any proteinaceous substance, such as venoms, as a therapeutic agent carries the potential danger of an allergic response to the agent by the patient. The use of scorpion venom as a therapeutic agent has not yet reached the clinical stage, nor has its chemical nature been completely elucidated. However, the presence of proteins or peptides in this venom has been indicated by electrophoretic analysis in this laboratory, using ninhydrin as an indicator.

In several preliminary experiments and in those reported here the venom of *Centruroides sculpturatus* has given no allergic response in animal experiments.

SUMMARY

The venom of *Centruroides sculpturatus* was tested for its ability to incite an allergic response in guinea pigs, using two standard methods. No allergic response was observed.

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Announcements

The American Foundation for Allergic Diseases

The American Foundation for Allergic Diseases, 801 Second Ave., New York, New York, announces postdoctoral fellowships in research and clinical allergy for two years each.

Stipend.—First year, $4,500.00; second year, $4,750.00; laboratory and travel expenses for two-year period, $750.00.

Requirements.—Candidates must be graduates of approved medical schools and must have completed one or two years of the graduate training required as a preliminary to certification by the Boards of Internal Medicine or Pediatrics; they are to divide their time between research and clinical training, and in the second year 10 or 15 per cent of a candidate's time might be devoted to teaching. The respective institutions have taken the necessary steps to have this training credited toward the subspecialty in allergy by the Board of Internal Medicine and the Board of Pediatrics.

Requests for applications should be sent directly to one of the following, in whose field the candidate would like to work:

Dr. Colin M. MacLeod,
Professor of Research Medicine,
University of Pennsylvania,
820 Maloney Clinic,
36th and Spruce Streets,
Philadelphia 4, Pennsylvania.

or

Dr. Herman N. Eisen,
Professor of Medicine (Dermatology),
Washington University School of Medicine,
600 South Kingshighway,
St. Louis 10, Missouri.

Applications should be filed no later than Dec. 15, 1957, with either of the above. Notification will be mailed to successful candidates by Feb. 1, 1958.

Chicago Allergy Society

The new officers of the Chicago Allergy Society are as follows:

President: Norman J. Ehrlich
President-Elect: Harold C. Wagner
Secretary-Treasurer: Israel A. Fond.

Michigan Allergy Society

The following are the officers of the Michigan Allergy Society:

President: E. Oskar Schreiber
Vice-President: Bernard Dickstein
Secretary-Treasurer: Milton J. Steinhardt
Executive Committee: Kenneth Mathews, Israel Wiener, Sidney Friedlaender.

Pittsburgh Allergy Society

The Pittsburgh Allergy Society elected the following officers on Sept. 9, 1957:

President: Philip Blank
Secretary-Treasurer: Macy I. Levine.

International Association of Allergology

A Nomenclature Committee has been nominated to make a report which will be discussed at the third international congress of the I.A.A. in Paris, France, in October, 1958.

The members of the Committee cordially invite their colleagues of the I.A.A., physicians, mycologists, bacteriologists, immunologists, and all scientists interested in allergy to collaborate by sending their suggestions to the Committee.

The members of the Committee are:

Dr. Guido Ruiz Moreno (Chairman), Berutti 2895, Buenos Aires, Argentina.
Dr. Joseph Harkavy, 1165 Park Ave., New York, N. Y.
Dr. Umberto Serafini, Via Guisciardini, Florence, Italy.
Dr. H. Stork, Talstrasse 41, Zürich, Switzerland.
Dr. J. M. Gutman, 14 Street of the Prophets, Jerusalem, Israel.

Suggestions made in English may be sent to Dr. Harkavy, those in French and Italian should be sent to Dr. Serafini, those in German should be sent to Dr. Stork, and those in Spanish and Portuguese should be directed to Dr. Ruiz Moreno.

Third International Congress of Allergology
Paris, France
Oct. 19-26, 1958

(Sponsored by International Association of Allergology and French Allergy Association)

Program

1. Symposia on asthma, emphysema, immunology, dermatology, socioeconomic aspects, drug allergy, etc.

2. Sectional previously *unpublished* papers, limited to ten minutes, on any phase of allergy. Send title, 200 word abstract, double-spaced, in triplicate, on thin paper to Dr. S. M. Feinberg, President, I. A. A., 303 E. Chicago Ave., Chicago 11, Illinois (for Americans), or to Dr. B. N. Halpern, Chairman of Organizing Committee, 197 Blvd. St. Germain, Paris VII, France (for non-Americans).

3. Round-table small group luncheon conferences on selected subjects led by international authorities.

4. Possible pre- or post-convention tours.

Session on Allergy of the American Medical Association

Readers are invited to submit manuscripts for the program of the Session on Allergy of the American Medical Association which will be held in San Francisco in June, 1958. Abstracts of 250 words should be submitted by Jan. 1, 1958, to the secretary of the Session, Dr. R. Dale Dickson, 403 National Reserve Bldg., Topeka, Kansas.

Books Received

Psychosomatic Medicine. A Clinical Study of Psychophysiologic Reactions. By Edward Weiss, M.D., Professor of Clinical Medicine, Temple University Medical Center, and O. Spurgeon English, M.D., Professor and Head of Department of Psychiatry, Temple University Medical Center. Third Edition. 557 pages, with illustrations. Philadelphia and London, 1957, W. B. Saunders Company. Price, $10.50.

The Dermatologist's Handbook. By Ashton L. Welsh, M.S., M.D., Assistant Professor of Dermatology and Syphilology, University of Cincinnati College of Medicine. 427 pages, Springfield, Illinois, 1957, Charles C Thomas, Publisher. Price, $15.00.

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